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A THEORETICAL STUDY OF CHOLINERGIC ACTION

by

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ABSTRACT

Theoretical methods for determining the components of the total interaction energy for the interaction of closed shell molecules are discussed. Expressions for the electrostatic and the polarization contributions based on perturbed self consistent field theory are presented. The dispersion energy is evaluated using the bond polarizability method. The overlap contributions are determined using a method based upon the electron gas approximation.

Experimental findings pertaining to cholinergic synaptic transmission and the action of cholinomimetic drugs are reviewed. Models for the active site of cholinergic receptors are examined and the probable mode of action of cholinomimetic agents is discussed. The potency of agonists is suggested to be related to the stability of the agonist-receptor complex. Factors that may affect the stability are discussed.

Calculated results pertaining to the interaction energy curves for the linear approach of select agonists to hypothetical receptor components are presented. The relative order of the respective binding energies and binding separations are established. The force of attraction of several agents to the receptor components is determined from the interaction energy curves for the rotation of each agent about its quaternary nitrogen center.

The implications of these findings for the postulated mode of action of cholinomimetic agents are examined.

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I. INTRODUCTION

Since the advent of approximate quantum mechanical methods based on the self consistent field (SCF) theory, it has become possible to calculate certain properties of molecular systems with some degree of accuracy. In the case of smaller molecules *ab initio* calculations are feasible, but for larger systems recourse is generally taken to semiempirical formulations of the theory.

The application of these methods in the study of the properties and functions of biomolecular systems is justifiable for a number of reasons, the foremost being the relative importance of this area of research for society. In addition the theoretical approach may be used to investigate phenomena that cannot readily be examined by experimental means. This is particularly true for the interaction of ligand molecules with specific sites on macromolecules.

In spite of the wealth of data that is available on the interaction of drugs with pharmacological receptors, the set of molecular events that are responsible for the pharmacological potency of these compounds have not been determined. The fact that these processes presumably do not involve a change in covalent bonding has led to the belief that quantum mechanical calculations may be particularly suited for investigating drug-receptor interactions.

In this study the binding of certain agents at cholinergic

receptors will be examined using a method based on perturbed SCF theory. In Chapter II the theoretical foundations of the approach will be discussed. Chapter III presents an outline of the relevant experimental findings. In Chapter IV models for the receptor binding site will be proposed. Chapter V deals with the results of the quantum mechanical calculations and in Chapter VI the implications of the results for the study of drug-receptor interactions are discussed.

II. THEORETICAL METHODS

Introduction

The application of quantum mechanical methods to the investigation of biochemical processes has largely involved two different approaches. On the one hand the focus has been on the determination of certain quantities, such as the dipole moments and the charge distributions of relevant systems of interest,that can be used to characterize the nature of the processes. On the other hand, where there is no question concerning the type of interactions involved, the aim has generally been to determine the energy changes taking place.

In the case of drug-receptor interactions the lack of knowledge about the receptor binding region has in the past limited the scope of the theoretical studies to the purely analytical level of application. The most commonly used procedure in the investigation of structure-activity relationships has been to calculate several quantities of interest, and to subsequently carry out a reduction analysis in order to establish possible parallel trends with the activity of a series of drugs. The underlying hope is presumably that the findings will reveal the specific molecular features an agent must possess to be pharmacologically active.

In recent years considerable progress has been made in the experimental investigation of phenomena related to drug action and nervous transmission. It now seems possible to carry out

a more direct quantum mechanical study of the processes involved. The most useful quantity for these purposes would be the free energy change associated with a given molecular event. However, due to the great difficulty involved in determining environmental effects, such as hydration and the presence of ionic surroundings, it will only be feasible to calculate the change in the internal energy for the binding processes.

The method used in this work is based upon the application of perturbation theory to SCF theory in a semiempirical formulation.

SCF Theory

The time-independent Schrödinger equation

$$H\Psi_r = E_r\Psi_r$$

is believed to be adequate for the description of many chemical and physical properties of molecular systems to a high degree of accuracy. The quantity E_r represents the energy eigenvalue corresponding to the state function Ψ_r, and the Hamiltonian operator (in atomic units) is

$$H \equiv \sum_{i}^{n} [-\frac{1}{2}\nabla_i^2 - \sum_{a}^{N} \frac{Z_a}{R_{ia}} + \frac{1}{2}\sum_{j}^{n}{}' \frac{1}{r_{ij}}]$$

where n is the number of electrons, N is the number of nuclei, ∇^2 is the Laplacian operator, Z_a is the nuclear charge, R_{ia}

is the electron-nuclear distance, and r_{ij} is the interelectronic distance.

The state functions Ψ_r are functions of the electronic and spin coordinates. The Pauli principle requires that an interchange of any two of these variables must cause a change in the sign of Ψ_r.

One may assign to each electron a function ψ_k, called a molecular spin-orbital, which depends on the space and spin coordinates of that electron and which can further be factored into a space part ϕ_k and a spin part η_k, i. e., for the i th electron

$$\psi_k(r_i, s_i) = \phi_k(r_i)\eta(s_i).$$

The set of n molecular orbitals $\{\psi_k\}$ are chosen to be orthonormal, so that

$$<\phi_k|\phi_\ell> = \delta_{k\ell}.$$

It is possible to approximate a given state function Ψ_r by an antisymmetrized product of molecular spin-orbitals, which can conveniently be expressed in terms of a Slater determinant

$$\Phi_r = \frac{1}{\sqrt{n!}} \begin{vmatrix} \psi_1(1) & \psi_2(1) & \cdots & \psi_n(1) \\ \psi_1(2) & \psi_2(2) & \cdots & \psi_n(2) \\ \cdots & \cdots & \cdots & \cdots \\ \psi_1(n) & \psi_2(n) & \cdots & \psi_n(n) \end{vmatrix} .$$

The electronic energy of a closed shell configuration is

then given by

$$E = \langle\Phi_o|H|\Phi_o\rangle$$

$$= \sum_k [2h_k + \sum_\ell (2J_{k\ell} - K_{k\ell})]$$

where the one electron integrals h_k, the coulomb integrals $J_{k\ell}$, and the exchange integrals $K_{k\ell}$ are defined as

$$h_k = \langle\phi_k(i)|h|\phi_k(i)\rangle = \langle\phi_k(i)|-\frac{1}{2}\nabla_i^2 - \sum_a \frac{Z_a}{R_{ia}}|\phi_k(i)\rangle$$

$$J_{k\ell} = \langle\phi_\ell|J_k|\phi_\ell\rangle = \langle\phi_k|J_\ell|\phi_k\rangle = \int \frac{\phi_k^*(i)\phi_\ell^*(j)\phi_k(i)\phi_\ell(j)}{r_{ij}} d\tau_i d\tau_j$$

$$K_{k\ell} = \langle\phi_\ell|K_k|\phi_\ell\rangle = \langle\phi_k|K_\ell|\phi_k\rangle = \int \frac{\phi_k^*(i)\phi_\ell^*(j)\phi_k(j)\phi_\ell(i)}{r_{ij}} d\tau_i d\tau_j$$

If one applies the variation principle to minimize the energy one obtains the coupled equations known as the Hartree-Fock SCF-equations, which, upon a unitary transformation within the set $\{\phi_k\}$, become

$$F\phi_k = \epsilon_k\phi_k$$

where the Fock operator is defined as

$$F \equiv h + \sum_\ell (2J_\ell - K_\ell) .$$

Generally, for molecular systems, it is necessary to approximate the molecular orbitals ϕ_k in terms of a linear combination of a set of m basis functions $\{\chi_q\}$, where $m \geq \frac{n}{2}$,

so that

$$\phi_k = \sum_q^m \chi_q c_{qk}.$$

The Slater orbitals [1] are most often used as basis functions. They are defined by

$$\chi_{n\ell m}(r, \theta, \phi) = R_n(r)\Theta_{\ell,|m|}(\theta)\Phi_m(\phi),$$

where $R_n(r)$, $\Theta_{\ell,|m|}(\theta)$, and $\Phi_m(\phi)$ are assumed to be normalized, and n, ℓ, and m are the three quantum numbers. The radial function $R_n(r)$ has the form $r^{n-1}\exp\{-\zeta r\}$ where ζ is the orbital exponent; $\Theta_{\ell,|m|}(\theta)$ represents the associated Legendre polynomials, and $\Phi_m(\phi)$ is $\exp\{-im\phi\}$.

Substitution of the expansion into the Hartree-Fock equations yields the Roothaan equations [2], which may be expressed in matrix form as

$$\underset{\sim}{F}\ \underset{\sim}{C} = \underset{\sim}{S}\ \underset{\sim}{C}\ \underset{\sim}{\varepsilon}.$$

Without loss of generality it may be assumed that $\underset{\sim}{\varepsilon}$ is a diagonal matrix, with real diagonal elements ε_i, which are called the orbital energies. The column vectors of the coefficient matrix $\underset{\sim}{C}$ corresponding to the $\frac{n}{2}$ lowest elements ε_i represent the occupied orbitals, while the remaining $(m - \frac{n}{2})$ vectors represent the virtual orbitals. The elements of the overlap matrix $\underset{\sim}{S}$ are

$$S_{qr} = \langle\chi_q|\chi_r\rangle,$$

and the elements of the Fock matrix $\underset{\sim}{F}$ are

$$F_{qr} = h_{qr} + \sum_{s}\sum_{t} P_{st}[(qr|st) - \frac{1}{2}(qs|rt)]$$

where

$$h_{qr} = \langle\chi_q|h|\chi_r\rangle$$

and

$$(qr|st) = \int \frac{\chi_q^*(i)\chi_s^*(j)\chi_r(i)\chi_t(j)}{r_{ij}} d\tau_i d\tau_j \ .$$

The elements of the bond order matrix P are

$$P_{st} = 2 \sum_{k}^{occ} c_{sk}c_{tk}.$$

The corresponding diagonal elements are the partial charges

$$q_s = P_{ss} = 2 \sum_{k}^{occ} c_{sk}^2.$$

In practice one adopts an iterative procedure to solve the n coupled equations.

The total electronic energy is given by

$$\begin{aligned} E &= \sum_{k} (h_{kk} + \varepsilon_k) \\ &= 2 \sum_{k} h_{kk} + \sum_{k}\sum_{\ell} (2J_{k\ell} - K_{k\ell}) \\ &= \sum_{s}\sum_{t} P_{st}\{h_{st} + \frac{1}{2} \sum_{q}\sum_{r} P_{qr}[(st|qr) - \frac{1}{2}(sq|tr)]\}. \end{aligned}$$

According to Koopman's theorem [3] the ionization potential I of a closed shell molecule is equal to the negative of the orbital energy ε_n corresponding to the highest occupied orbital,

i. e.

$$I = -\varepsilon_n$$

Approximate Formulations

To reduce the cost of the calculations for large systems, numerous formulations involving approximations to the original SCF theory have been developed.

An assumption that is common to the all-valence-electron methods (AVE) is that the inner electrons are unaffected by bonding, and can thus be considered to constitute an undisturbed core. The various formulations in this scheme differ in the explicit expressions used for the elements of the Fock matrix.

The simplest approximate version is the extended Hueckel theory (EHT) [4, 5, 6]. The diagonal elements of the matrix corresponding to F are taken to be equal to the negative of the valence state ionization potential for the orbital in question. The off-diagonal elements are evaluated using a variety of formulas, of which two frequently encountered examples are

$$H_{ij} = \frac{KS_{ij}}{2} (H_{ii} + H_{jj}) ,$$

and

$$H_{ij} = K'S_{ij} (H_{ii} H_{ij})^{\frac{1}{2}} ,$$

where the constants K and K' depend on the type of integrals being evaluated.

The distinguishing feature of the CNDO (complete neglect

of differential overlap) method [7, 8, 9] is the ZDO (zero differential overlap) approximation, i. e. the neglect of the overlap elements between different basis functions

$$\chi_q \chi_r d\tau = 0 \quad (q \neq r)$$

in the electronic repulsion integrals and in the overlap integrals

$$(qr|st) = 0 \qquad (q \neq r \text{ or } s \neq t)$$

$$S_{qr} = 0 \qquad (r \neq q).$$

In the hope of partly compensating for the neglected terms, some of the retained integrals are evaluated using parameters derived from experiment. To assure that the value of the two-electron integrals is invariant to rotations of the molecule it is common to use Slater-type s-orbitals for the p-functions.

In the modified version of CNDO known as the INDO (intermediate neglect of differential overlap) method, the electron repulsion integrals of the form (qr|qr) are not neglected if χ_q and χ_r are centered on the same atom. Various formulations of this general approach exist [10, 11], which differ in the degree and the type of the parametrization used for the integrals.

In this work use will be made of a method due to Fraga and Carbo [12]. The matrix elements of the Fock operator are defined by

$$F_{rr} = H_{rr} + \frac{1}{2} q_r J_{rr} + \sum_{s \neq r} P_{rs} J_{rs}$$

$$F_{rs} = H_{rs} - \frac{1}{2} P_{rs} J_{rs}$$

where the subindices denote the valence electron orbitals (for the calculations in this work, 1s for H and 2s, 2p for the atoms of the first row).

The one-electron terms are defined by

$$H_{rr} = U_{rr} - \sum_a{}' Z_a V_{rr;a} = T_{rr} - \sum_a Z_a V_{rr;a}$$

$$H_{rs} = \frac{1}{2} S_{rs}(F_{rr} + F_{ss})$$

with

$$V_{rr;a} = \langle\chi_r(i)|\frac{1}{R_{ia}}|\chi_r(i)\rangle, \quad T_{rr} = \langle\chi_r(i)|-\frac{1}{2}\nabla_i^2|\chi_r(i)\rangle.$$

where the summation a extends over all the centers in the system (with the core charges Z_a).

The integrals S_{rs} and J_{rs} are evaluated using the formulation of Roothaan [13]. The one-electron, one-center terms U_{rr} (including the kinetic and nuclear attraction energies), are calculated from the expressions

$$U_{2s,2s} = \frac{1}{2}(\frac{1}{3}\zeta_{2s} - Z)\zeta_{2s}$$

$$U_{2p,2p} = \frac{1}{2}(\zeta_{2p} - Z)\zeta_{2p}$$

where Z represents the effective nuclear charge of the atom under consideration, corrected by the number of inner electrons, and ζ are the orbital exponents.

Due to the explicit use of 2p functions within this approach at the CNDO level, rotational invariance is not preserved. This

is justifiable on the basis of the work of Dewar [14], and also because the orientational property of the 2p orbitals may be of importance in the calculation of intermolecular interactions for which the AVE SCF results are to serve as input.

Intermolecular Interactions

In discussing the interaction of two closed shell molecules it is common to decompose the total interaction energy into a number of component energies. The term "long range" interactions is used to collectively denote electrostatic, polarization and dispersion contributions, while the term "short range" interactions is reserved for contributions that increase rapidly with the overlap - in particular, effects due to the Pauli principle.

(1) Long Range Interactions.

The case of the interaction of two non-overlapping systems is conventionally treated within the framework of perturbation theory. Longuet-Higgins [15] has presented a general formulation of the problem.

Given two systems S and S' with the ground state wave functions $|a\rangle$ and $|a'\rangle$, and the excited state wave functions $|b\rangle$ and $|b'\rangle$, respectively, for which the interaction Hamiltonian U is expressible as the difference between a zero order Hamiltonian H_o for the separate systems and the total Hamiltonian H, one may derive the following perturbation expansion for the interaction energy to the second order

$$E^{INT} = \langle aa'|U|aa'\rangle - \sum_{b'}{}' \frac{|\langle aa'|U|ab'\rangle|^2}{E_{b'}-E_{a'}}$$

$$- \sum_{b}{}' \frac{|\langle aa'|U|ba'\rangle|^2}{E_b-E_a} - \sum_{b}\sum_{b'} \frac{|\langle aa'|U|bb'\rangle|^2}{E_b-E_a+E_{b'}-E_{a'}} + \ldots$$

The first term is the electrostatic interaction energy, the second and third terms represent the energy due to the polarization of systems S' and S respectively, and the last term represents the dispersion energy.

A number of formulations for the calculation of the electrostatic and the polarization energies using a perturbed SCF method have been put forth, for both one-electron [16, 17, 18, 19, 20, 21, 22, 23] and two-electron [24] perturbations. In this study an approach adapted to the AVE SCF method of Fraga and Carbo will be presented.

To estimate the dispersion energy for the interaction of large systems it is generally necessary to utilize the available empirical values for the polarizability of suitable molecular segments [25, 26, 27, 28]. For the calculations reported in this study the bond polarizability approximation was used [29]. The dispersion energy is given by

$$E_{disp} = -\frac{1}{4}\frac{I_1 I_2}{I_1+I_2}\sum_{i=1}^{b_1}\sum_{j=1}^{b_2}\frac{1}{r_{ij}^6}\,[6\alpha_i^T\alpha_j^T + \alpha_i^T\delta_j\{3(\vec{\alpha}_j^L\cdot\vec{r}_{ij})^2 + 1\}$$

$$+ \alpha_j^T\delta_i\{3(\vec{\alpha}_i^L\cdot\vec{r}_{ij})^2 + 1\} + \delta_i\delta_j\{3(\vec{\alpha}_i^L\cdot\vec{r}_{ij})(\vec{\alpha}_j^L\cdot\vec{r}_{ij})$$

$$- (\vec{\alpha}_i^L\cdot\vec{\alpha}_j^L))\}^2],$$

where the summations extend over the number of bonds b_1 and b_2 in molecules 1 and 2 respectively, I_1 and I_2 are the ionization potentials, α_i^T and α_i^L are the transverse and longitudinal polarizabilities of the i^{th} bond, r_{ij} is the separation of the midpoints of the i^{th} and j^{th} bonds, $\vec{\alpha}_i^L$ and $\vec{r}_{ij}$ are the unit vectors pointing respectively along the i^{th} bond and the line joining the midpoints of the i^{th} and j^{th} bonds, and $\delta_i = \alpha_i^L - \alpha_i^T$.

(2) Short Range Interactions.

There have been numerous attempts to formulate a perturbation approach suitable for calculating the short range as well as the long range interaction energies (see [30] for a review). It appears, however, that no generally applicable method for the study of the interaction of large systems has yet been put forth.

On the other hand, direct variational calculation of interaction energies usually yields unsatisfactory results, since the error involved in determining these quantities as a difference of two total energies is generally quite large [31].

A novel method for determining the short range components for the interaction of closed shell atomic systems was proposed by Gordon and Kim [32, 33], and modified and corrected by Rae [34]. The underlying assumption for the approach is that the electron distribution in the overlap region between the interacting systems can be treated locally as a uniform electron gas. Expressions for the energy density of a uniform electron gas, including the correlation energy density, are available.

The Hartree-Fock portion of the energy density of an electron gas of density ρ is given as the sum of a kinetic energy and an exchange energy contribution per electron

$$E_{HF}(\rho) = E_k(\rho) + E_x(\rho)$$

where

$$E_k = \frac{3}{10}(3\pi^2)^{\frac{2}{3}}\rho^{\frac{2}{3}}$$

and

$$E_x = -\frac{3}{4}(3/\pi)^{\frac{1}{3}}\rho^{\frac{1}{3}}[1 - \frac{8}{3}\delta x + 2\delta x^2 - \frac{1}{3}\delta x^4].$$

The term in brackets represents a correction for self-exchange where

$$\delta x \approx (4N)^{-\frac{1}{3}}$$

and N is the number of electrons in the total interacting system.

Expansions for the correlation energy density per electron are available for three different density ranges [35]

$$E^{\ell}_{CORR}(r_s) = -0.438r_s^{-1} + 1.325r_s^{-\frac{3}{2}} - 1.47r_s^{-2} - 0.4r_s^{-\frac{5}{2}} \quad (r_s > 10)$$

$$E^{i}_{CORR}(r_s) = 0.01898\ell n r_s - 0.06156 \quad (0.7 < r_s < 10)$$

$$E^{h}_{CORR}(r_s) = 0.0311\ell n r_s - 0.048 + 0.009 r_s \ell n r_s - 0.01 r_s \quad (r_s < 0.7)$$

where the radius r_s is related to the density ρ by

$$\frac{4\pi}{3}\rho r_s^3 = 1.$$

If $\rho_A(\vec{r})$ and $\rho_B(\vec{r})$ are the electron distributions of the two interacting systems A and B, the total energy due to short

range effects - which will be termed the overlap energy (E_{OVLP}) - is given by

$$E_{OVLP} = \int d\vec{r}\{[\rho_A(\vec{r}) + \rho_B(\vec{r})]E(\rho_A + \rho_B) - \rho_A(\vec{r})E(\rho_A) - \rho_B(\vec{r})E(\rho_B)\}$$

with

$$E(\rho) = E_{HF}(\rho) + E_{CORR}(\rho).$$

For the calculations reported in this study the molecular electron distributions were determined using the AVE SCF method of Fraga and Carbo. The local density is given as

$$\rho(\vec{r}) = \sum_s q_s |\chi_s(\vec{r})|^2$$

where the summation runs over all basis functions on all centers (including the 1s-functions which are assumed to be completely localized).

The integration was carried out using a standard three dimensional numerical technique [36].

(3) Total Interaction Energy.

A summary can now be given of the component energies that will be included in the calculation of the total interaction energy E^{INT}, where

$$E^{INT} = E_{EL} + E_{POL} + E_{DISP} + E_{OVLP}.$$

The electrostatic and the polarization energies (E_{EL} and E_{POL}) will be determined using an approximate perturbed SCF formulation. The dispersion energy will be estimated using the bond polarizability approximation. The overlap contribution E_{OVLP}

will be determined using the method based on the electron gas approximation.

Perturbed SCF-Theory

An SCF formulation for determining the electrostatic and the polarization energies of two interacting closed shell molecules (A and B) will now be presented [37, 38]. The corresponding SCF equations are

$$F\phi_n = \phi_n\theta_n$$

where F is the total Fock operator, defined in terms of the orbitals ϕ_n, that extend over the whole interacting system. At large separations between the two molecules, the set $\{\phi\}$ decomposes into the two sets of perturbed orbitals $\{\phi_A\}$ and $\{\phi_B\}$ for the individual molecules.

The unperturbed SCF equations for molecule A are

$$F_A^{(0)}\phi_{A,i}^{(0)} = \phi_{A,i}^{(0)}\varepsilon_{A,i}^{(0)}$$

where

$$F_A^{(0)} = -\frac{1}{2}\nabla^2 + h_A + \sum_{i}^{OCC(A)} I_{A,ii}^{(0,0)}$$

with

$$h_A = -\sum_{a}^{(A)} \frac{Z_a}{R_a}$$

and

$$I_{A,ii}^{(0,0)} = (2J_{A,ii}^{(0,0)} - K_{A,ii}^{(0,0)})$$

defined in terms of the unperturbed orbitals $\phi_{A,i}^{(0)}$.

The perturbed equations for system A in the presence of molecule B, defined in terms of the perturbed orbitals, are

$$F_{p(A)}\phi_{A,i} = \phi_{A,i}\varepsilon_{A,i}$$

where

$$F_{p(A)} = F_A + f_B = -\frac{1}{2}\nabla^2 + f_A + \sum_{i}^{OCC(A)} I_{A,ii} + f_B$$

with

$$f_B = h_B + \sum_{\ell}^{OCC(B)} I_{B,\ell\ell}$$

and

$$h_B = -\sum_{b}^{(B)} \frac{Z_b}{R_b}$$

$$I_{B,\ell\ell} = (2J_{B,\ell\ell} - K_{B,\ell\ell}).$$

(1) Perturbation Theory.

One may formulate the problem of determining the perturbed orbitals ϕ_A and the orbital energies ε_A within the framework of the Rayleigh-Schrödinger perturbation theory. The presence of molecule B can be considered to represent a perturbation acting on system A. Introducing the perturbation parameter λ one may write the perturbed equations as

$$F_{p(A)}\phi_{A,i} = (F_A + \lambda f_B)\phi_{A,i} = \phi_{A,i}\varepsilon_{A,i}.$$

At the outset the perturbation term will be defined using the unperturbed orbitals of system B, i. e.,

$$f_B \equiv f_B^{(0)} = h_B + \sum_{\ell}^{OCC(B)} I_{B,\ell\ell}^{(0,0)}.$$

One may further carry out the expansions

$$\phi_{A,i} = \sum_n \phi^{(n)}_{A,i}\lambda^n \qquad \varepsilon_{A,i} = \sum_n \varepsilon^{(n)}_{A,i}\lambda^n .$$

Substituting these expressions into the perturbed equations for A and equating equal powers of λ one obtains

$$F^{(0)}_A \phi^{(0)}_{A,i} = \varepsilon^{(0)}_{A_{ii}} \phi^{(0)}_{A,i}$$

$$(F^{(0)}_A - \varepsilon^{(0)}_{A,i})\phi^{(1)}_{A,i} = -(f_B + 2 \sum_j^{OCC(A)} I^{(0,1)}_{A,jj} - \varepsilon^{(1)}_{A,i})\phi^{(0)}_{A,i}$$

$$(F^{(0)}_A - \varepsilon^{(0)}_{A,i})\phi^{(2)}_{A,i} = -(f_B + 2 \sum_j^{OCC(A)} I^{(0,1)}_{A,jj} - \varepsilon^{(1)}_{A,i})\phi^{(1)}_{A,i}$$

$$+ [\varepsilon^{(2)}_{A,i} - \sum_j^{OCC(A)} (I^{(1,1)}_{A,jj} + 2I^{(0,2)}_{A,jj})]\phi^{(0)}_{A,i}$$

One may expand each perturbation correction in terms of the unperturbed orbitals

$$\phi^{(n)}_{A,i} = \sum_k \phi^{(0)}_{A,k} a^{(n)}_{ki} .$$

Further introducing the decomposition

$$f_B \phi^{(n)}_{A,i} = \sum_k \sum_\ell \phi^{(0)}_{A,k} f_{B;A,k\ell} a^{(n)}_{\ell i}$$

where

$$f_{B;A,k\ell} = \langle\phi^{(0)}_{A,k}|f_B|\phi^{(0)}_{A,\ell}\rangle$$

one obtains, after left-multiplying the perturbation equations by $\langle\phi^{(0)}_{A,i}|$,

$$\varepsilon^{(1)}_{A,i} = f_{B;A,ii} + 2 \sum_j \sum_{k \neq j} I^{(0,0)}_{A,jkii} a^{(1)}_{kj}$$

$$\varepsilon^{(2)}_{A,i} = \sum_{k\neq i} f_{B;A,ik} a^{(1)}_{ki} + \sum_{j} \sum_{k\neq j} \sum_{\ell\neq j} I^{(0,0)}_{A,k\ell ii} a^{(1)}_{kj} a^{(1)}_{\ell j}$$

$$+ 2 \sum_{j} \sum_{k} I^{(0,0)}_{A,jkii} a^{(2)}_{kj} .$$

Substituting these expressions for the orbital energy corrections back into the original equations and left-multiplying by $<\phi^{(0)}_{A,k}|$ yields

$$a^{(1)}_{ki} = (\varepsilon^{(0)}_{A,i} - \varepsilon^{(0)}_{A,k})^{-1} f_{B;A,ki} \quad .$$

$$a^{(2)}_{ki} = (\varepsilon^{(0)}_{A,i} - \varepsilon^{(0)}_{A,k})^{-1} (\sum_{\ell\neq i} f_{B;A,k\ell} a^{(1)}_{\ell i} - \varepsilon^{(1)}_{A,ii} a^{(1)}_{ki}$$

$$+ 2 \sum_{j} \sum_{\ell\neq j} I^{(0,0)}_{A,j\ell kk} a^{(1)}_{\ell j} a^{(1)}_{ki}).$$

These coefficients must satisfy the orthonormality conditions

$$<\phi^{(0)}_{A,i}|\phi^{(1)}_{A,j}> + <\phi^{(1)}_{A,i}|\phi^{(0)}_{A,j}> = 0$$

$$<\phi^{(0)}_{A,i}|\phi^{(2)}_{A,j}> + <\phi^{(1)}_{A,i}|\phi^{(1)}_{A,j}> + <\phi^{(2)}_{A,i}|\phi^{(0)}_{A,j}> = 0$$

that is

$$a^{(1)}_{ij} + a^{(1)}_{ji} = 0$$

$$a^{(2)}_{ij} + \sum_{k\neq i,j} a^{(1)}_{ki} a^{(1)}_{jk} + a^{(2)}_{ji} = 0.$$

(2) Total Energy.

The total electronic energy for the two interacting systems is given by

$$E_e = \sum_{i}^{OCC} \langle\phi_i|H + F|\phi_i\rangle$$

$$= \sum_{i}^{OCC(A)} \langle\phi_{A,i}|H_A+F_A+\lambda f_B|\phi_{A,i}\rangle + \sum_{j}^{OCC(B)} \langle\phi_{B,j}|H_B+F_B+\lambda f_A|\phi_{B,j}\rangle$$

$$= \sum_{i}^{OCC(A)} \langle\phi_{A,i}|2H_A+ \sum_{k}^{OCC(A)} I_{A,kk} + \lambda(2h_B+ \sum_{\ell}^{OCC(B)} I_{B,\ell\ell})|\phi_{A,i}\rangle$$

$$+ \sum_{j}^{OCC(B)} \langle\phi_{B,j}|2H_B+ \sum_{m}^{OCC(B)} I_{B,mm} + \lambda(2h_A+ \sum_{n}^{OCC(A)} I_{A,nn})|\phi_{B,j}\rangle$$

where

$$H = H_A + H_B$$

$$= -\frac{1}{2}\nabla^2 + h_A + h_B.$$

Substituting the expressions for the perturbed orbitals derived above, the electronic energy for system A to second order is

$$E_{e,A} = E_A^{(0)} + E_A^{(1)} + E_A^{(2)}$$

where the unperturbed energy is given by

$$E_A^{(0)} = \sum_{i}^{OCC(A)} \langle\phi_{A,i}^{(0)}|H_A + F_A^{(0)}|\phi_{A,i}^{(0)}\rangle,$$

and the first and second order corrections are

$$E_A^{(1)} = \sum_{i}^{OCC(A)} (h_{B;A,ii} + f_{B;A,ii})$$

$$E_A^{(2)} = 2 \sum_{i}^{OCC(A)} \sum_{k}^{VIRT(A)} (h_{B;A,ik}+f_{B;A,ik})a_{ki}^{(1)}$$

$$= 2 \sum_{i}^{OCC(A)} \sum_{k}^{VIRT(A)} (h_{B;A,ik} + f_{B;A,ik}) \frac{f_{B;A,ki}}{(\varepsilon_{A,i}^{(0)} - \varepsilon_{A,k}^{(0)})} .$$

Corresponding expressions hold for system B.

The electrostatic interaction energy is given by

$$E_{EL} = E_A^{(1)} + E_B^{(1)} + \sum_{a}^{(A)} \sum_{b}^{(B)} \frac{Z_a Z_b}{R_{ab}}$$

where R_{ab} is the internuclear separation. The polarization component of the interaction energy is

$$E_{POL} = E_A^{(2)} + E_B^{(2)} .$$

(3) Approximate Formulation.

The perturbed SCF method used for the calculations reported in this study was designed to correspond in its essential features to the AVE SCF method of Fraga and Carbo.

Expanding the molecular orbitals in terms of a set of basis functions and introducing the ZDO approximation one obtains

$$E_A^{(1)} = \sum_{i}^{OCC(A)} \langle \phi_{A,i}^{(0)} | h_B + f_B | \phi_{A,i}^{(0)} \rangle$$

$$= \sum_{i}^{OCC(A)} \sum_{r}^{(A)} c_{A,ri}^2 \langle r | h_B + f_B | r \rangle$$

$$= \frac{1}{2} \sum_{r}^{(A)} q_{A,r} [2h_{B,rr} + \sum_{s}^{(B)} q_{B,s} (rr|ss)]$$

$$E_A^{(2)} = 2 \sum_{i}^{OCC(A)} \sum_{k}^{VIRT(A)} \langle \phi_{A,i}^{(0)} | h_B + f_B | \phi_{A,k}^{(0)} \rangle \langle \phi_{A,k}^{(0)} | f_B | \phi_{A,i}^{(0)} \rangle (\varepsilon_{A,i}^{(0)} - \varepsilon_{A,k}^{(0)})^{-1}$$

$$\approx \frac{2}{\Delta\varepsilon_{OV}} \sum_{i}^{OCC(A)} \sum_{k}^{VIRT(A)} \langle\phi_{A,i}^{(0)}|h_B+f_B|\phi_{A,k}^{(0)}\rangle\langle\phi_{A,k}^{(0)}|f_B|\phi_{A,i}^{(0)}\rangle$$

$$= \frac{2}{\Delta\varepsilon_{OV}} \sum_{i}^{OCC(A)} \sum_{k}^{VIRT(A)} \sum_{r}^{(A)} \sum_{s}^{(A)} c_{A,ri}c_{A,rk}c_{A,si}c_{A,sk}$$

$$[2h_{B,rr} + \sum_{t}^{(B)} q_{B,t}(rr|tt)][h_{B,ss} + \sum_{u}^{(B)} q_{B,u}(ss|uu)]$$

$$= \frac{1}{2\Delta\varepsilon_{OV}} \sum_{r}^{(A)} \sum_{s}^{(A)} p_{A,rs}(2\delta_{rs}-p_{A,rs})[2h_{B,rr} + \sum_{t}^{(B)} q_{B,t}(rr|tt)]$$

$$[h_{B,ss} + \sum_{u}^{(B)} q_{B,u}(ss|uu)]$$

where $\Delta\varepsilon_{OV}$ represents the average occupied-virtual orbital excitation energy, and use has been made of the equality

$$2 \sum_{k}^{VIRT} c_{rk}c_{sk} = 2\delta_{rs} - 2 \sum_{i}^{OCC} c_{ri}c_{si} = 2\delta_{rs} - p_{rs}.$$

III. AN OUTLINE OF NEUROCHEMISTRY

Anatomy and Physiology of the Nervous System

The vertebrate nervous system is commonly divided into the peripheral network, containing efferent nerve fibers from the sense organs as well as afferent fibers which innervate muscles and viscera, and the central nervous system, which consists of the spinal chord and the brain. Aside from this anatomical distinction there are also two major functional divisions. One is the autonomic nervous system (ANS) which involves functions not requiring conscious activation, and the other is the somatic system.

There are two functional divisions within the ANS: the parasympathetic and the sympathetic systems. Each is composed of peripheral sensory fibers, central integrating areas, and peripheral motor preganglionic and postganglionic fibers. Functionally the parasympathetic system is generally linked with energy conserving, growth directed activities, while the sympathetic system is linked with energy expending activities.

Each nerve fiber consists of bundles of nerve cells (or neurones). The individual neurone is by no means a stereotype structural unit. However, in spite of great diversities of shape and function, there are a number of features all neurones have in common. Like other cells they are surrounded by a cell membrane, and they contain a soma, as well as those innercellular structures necessary for cell

functioning. Morphologically they differ in that they have certain elongations, called axons or dendrites depending on their function, which extend away from the cell body.

As a rule, the axonal endings of one nerve cell are in contact with the dendrites or cell bodies of other neurones. The axons of afferent neurones form similar junctions with muscles. These regions of contact are termed synapses. The membrane of the axon terminal is known as the presynaptic membrane, that of the adjacent dendrite, cell body, or muscle, as the postsynaptic membrane. The space between the presynaptic and postsynaptic membranes is called the synaptic cleft. In vertebrate species, the width of this cleft at synapses is 200 Å - 300 Å while at neuromuscular junctions it may be 500 Å - 1000 Å.

Axons are bathed in a fluid that contains the ions Na^+, K^+, Ca^{+2}, Mg^{+2}, and Cl^-. The interior of the axons is filled with the so called axoplasm, a gel which also contains all the ions listed as well as certain organic anions. Close to the presynaptic membrane there are clusters of small globular structures called vesicles. Often these are found imbedded in hollows in the membrane.

In an intact nerve the concentrations of the ions in the axon interior will not be the same as those in the external fluid. For example, in the case of the squid axon, the respective concentration ratios (out/in) are [39]:

$$[Na^+],\ 10:1;\ [K^+],\ 1:40;\ [Cl^-],\ 14:1.$$

To rationalize this fact it was found necessary to postulate an active transport mechanism, a so called "sodium pump", which ejects sodium ions from the axon interior [40]. The large concentration gradient leads to a potential gradient of approximately -60 mV across the axonal membrane of most neurones.

If a depolarizing potential larger than 20 mV is applied to the membrane in the resting state, one observes a rapid reversal of the potential difference, the interior of the axon momentarily becoming 50 mV more positive than the external fluid. This phenomenon, known as an action potential, is accompanied by the following events listed in the order of their occurrence [41]:

a) A large temporary increase in membrane conductivity to sodium ions resulting in a sudden influx of positive charge.

b) An increase in the conductivity to potassium ions, which serves to restore the potential difference of the resting state.

Once the action potential response has taken place in a certain region of the axon, small local circuits due to the migration of ions will cause it to spread into adjacent regions.

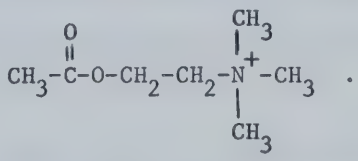
When the depolarization associated with the action potential reaches the presynaptic membrane, it causes a quantum of a compound contained in the vesicles to be released into the synaptic cleft. This transmitter substance rapidly diffuses to the postsynaptic membrane, where it can interact with special receptor sites to generate a change in potential.

Unlike the action potential, this postsynaptic potential change is not an all-or-none event. The greater the frequency of the action potentials reaching the presynaptic membrane, the more transmitter substance will be released, resulting in a corresponding graded change in the postsynaptic potential [42].

Since the average neurone makes many synaptic contacts, the effects of the various postsynaptic potentials must be integrated before an action potential is generated.

Acetylcholine

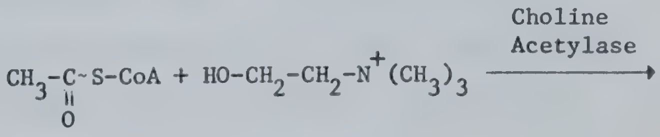
Acetylcholine (ACh) has been identified as the transmitter substance of all postganglionic parasympathetic neurones, as well as some postganglionic sympathetic neurones



$$CH_3-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-\overset{CH_3}{\overset{|}{\underset{CH_3}{\underset{|}{N^+}}}}-CH_3 \; .$$

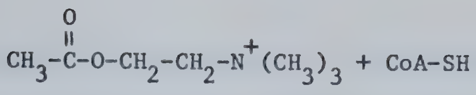
It is stored in the vesicles at a concentration of 150 mM, while its concentration in the axoplasm is only .4 mM.

ACh is believed to be synthesized in the axoplasm and then transported into the vesicles. The synthesis takes place according to the following pathway



$$CH_3-\underset{O}{\underset{\|}{C}}\sim S-CoA + HO-CH_2-CH_2-\overset{+}{N}(CH_3)_3 \xrightarrow{\text{Choline Acetylase}}$$

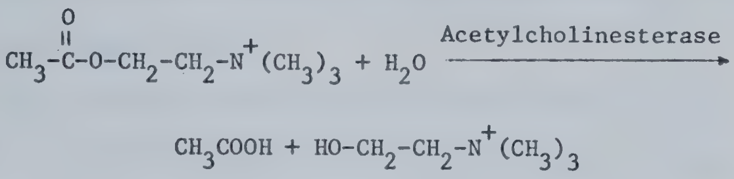
Acetyl-CoA Choline



$$CH_3-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3 + CoA-SH$$

Acetylcholine CoA

while the metabolic destruction occurs according to



$$CH_3-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3 + H_2O \xrightarrow{\text{Acetylcholinesterase}}$$

$$CH_3COOH + HO-CH_2-CH_2-\overset{+}{N}(CH_3)_3$$

Acetylcholinesterase (AChE), the enzyme that very rapidly hydrolyzes ACh, is present at the postsynaptic membrane but not in the axoplasm [43]. The active site contains an anionic site (probably glutamic acid), two basic groups (probably histidine residues), and an acetylatable serine group. It is believed that one of the basic residues is involved in the deacetylation, while the other supports the formation of the subsequent acetyl enzyme [44].

Upon the depolarization due to an action potential, a given vesicle may release a quantum of 100 - 10000 molecules of ACh [45]. These diffuse across the synaptic cleft to the postsynaptic membrane, where an interaction with specific receptor sites may occur, initiating a series of molecular events leading to a postsynaptic potential change. The minimum effective quantity of ACh is of the order of 10^{-17} to 10^{-16} moles [39]. It is rapidly hydrolyzed by the postsynaptic AChE, and the choline thereby produced may be transported back into the axon, and utilized in the synthesis of further ACh.

The molecular mechanism leading to a postsynaptic potential change is clearly distinguishable from that involved in maintaining the action potential. It is known to operate over a wide range of membrane potential, and the ionic changes involved occur very rapidly [46].

The interaction of ACh with the acetylcholine receptor (AChR) is believed to cause an opening of pores or channels through the membrane, which leads to a permeability change to at least two ions, one of which must be Na^+ [47].

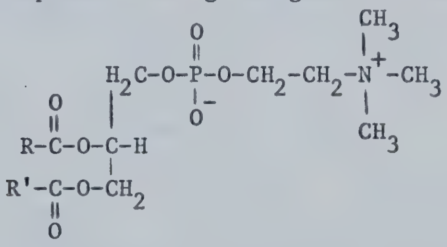
The extracellular concentration of Ca^{+2} plays a critical role in the maintainance of the cell excitability. Both in nerve and muscle preparations, a raising of the Ca^{+2}concentration leads to an increase in the resting potential, while a lowering of the Ca^{+2} level leads to a lowering of the resting potential and to spontaneous excitability [48]. This effect is primarily due to a reduction of the membrane Na^+ conductance with increasing Ca^{+2} concentration, and has been attributed to the competition between Na^+, K^+ and Ca^{+2} for fixed negative charges on the cell membrane [49]. An allied phenomenon that can be similarly explained is the reduction of the conductance change produced by ACh with an increase in the external Ca^{+2} concentration.

Membranes

In order to attempt to rationalize the excitability of natural membranes it will be useful to review briefly the results of investigations on the chemical composition, the organization of membrane components, and the physical state of membranes.

Although biological membranes vary greatly in composition, lipids, proteins, inorganic ions, and water are present in all natural membranes. The most commonly occurring lipids are phospholipids such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidic acid.

The bipolar compounds having the general formula



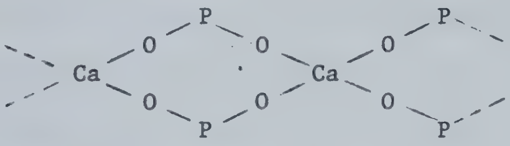
(R, R' = saturated or unsaturated aliphatic chain)

collectively are referred to as phosphatidylcholine. Since the polar portion of these lipids closely resembles the ACh molecule, it has been proposed that phosphatidylcholine is involved in the mechanism for membrane excitation by ACh [50, 51].

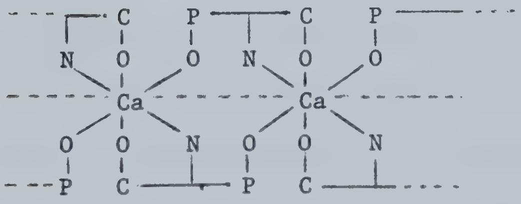
There is now ample evidence that the basic structure preserving unit of biological membranes is a lipid bilayer [52, 53, 54, 55]. The hydrophilic parts of the polar lipids constitute the surfaces of the membrane, while their hydrophobic exten-

sions are buried in the membrane interior. The width of the bilayer is approximately 45 Å.

Several studies indicate that Ca^{+2} has an ordering effect on the lipid polar groups [56, 57, 58]. The ion is believed to form a link between the negatively charged lipid phosphate substituents, thereby decreasing their mutual repulsion. Although there is some evidence that Ca^{+2} does not form such a complex with phoshatidylcholine [59], the following models for the complexing of phosphatidic acid and phosphatidylserine by Ca^{+2} have been proposed [60]



(phosphatidic acid)



(phosphatidylserine)

In addition it was found that Na^{+} and K^{+} are much less effective than Ca^{+2} in complexing lipid phases. Furthermore, these monovalent ions actually antagonize the action of Ca^{+2} [61].

Of the polyvalent cations that are able to compete with Ca^{+2} for the binding sites on phospholipids, UO_2^{+2} has been singled out as particularly active [62]. This is of importance since UO_2^{+2} is known to have a profound effect on a number of membrane mediated processes. In particular it was found that like Ca^{+2}, it increases the threshold for action potential generation in muscles, and similarly tends to decrease the sensitivity to ACh [63]. Moreover both Ca^{+2} and UO_2^{+2} can competitively inhibit the membrane depolarization produced by the ACh analogue, carbamylcholine [64].

All this strongly suggests that phospholipids are critically involved in the AChR, and that the depolarization produced by ACh and its analogues at synapses may involve the displacement of membrane complexed Ca^{+2}.

Both enzymes as well as structural proteins may be present in natural membranes. Membrane proteins that lie close to the surface of the bilayer are known as extrinsic proteins, those that are imbedded in the membrane or that extend all the way through it are called intrinsic proteins. Due to the fluidity of the lipid bilayer, both lipid and protein molecules are free to move transversely, when constraints do not prevent it [65]. However there is evidence that the formation of supramolecular aggregates of both extrinsic and intrinsic proteins may fix their spatial distribution in certain membranes [66].

Also it has been found that the so called boundary lipids in the proximity of intrinsic proteins are relatively immobilized [67].

The study of lipid enclosed globules, called vesicles or liposomes, has shed some light on the permeability properties of bilayers. It was found that the rate of diffusion of small monovalent cations across the membrane is influenced by the charge of the polar head groups of the membrane phospholipids [68]. Making the interfacial potential negative increases the rate of diffusion, making it more positive decreases it. However, this simplistic electrostatic dependence was not found to hold for anions. Divalent cations have the effect of promoting the transport of monovalent ions.

Artificial membranes reconstituted from solutions of lipids have also been used to gain insight into the nature and function of biomembranes [69, 70, 71]. The high electrical resistance of these membranes supports the view that they essentially consist of a continuous lipid phase. A number of studies have shown that the cation conductivity of these reconstituted membranes can be modified in the presence of certain appropriate macromolecules.

Antibiotics such as nonactin and valinomycin act as carriers which can transport select cations across the membranes [72, 73]. These globular macromolecules are nonpolar on the surface but contain a polar interior. They are thought to form a one-to-one complex with cations. Since the hydrocarbon core of the lipid bilayer presents a barrier to cation mobility the antibiotic-

cation complex is believed to "dissolve" in the membrane interior, and to release the cation at the opposing face.

The addition of gramicidin A, a lipid soluble, helical protein, to a lipid membrane has the effect of increasing the membrane conductance of ions nonspecifically [74]. Furthermore the permeability to water and small non-electrolytes is also increased. It has therefore been postulated that this protein is able to form pores in the membrane.

Finally it was found that the electric properties of a reconstituted membrane, to which a minute amount of a proteolipid extracted from the electric organ of the electric eel has been added, are greatly altered in the presence of ACh. This effect is actually inhibited if drugs that are known to block the AChR are added [75].

In summary it may be said that the conductivity of a lipid bilayer to cations may be influenced by the specific lipids present, and that increases in conductance may be observed in the presence of certain macromolecules. In addition there exists a macromolecule which alters the electrical properties of the bilayer in the presence of ACh, and which may in fact be identical to the AChR.

Nature of the Cholinergic Receptor

Recently there have appeared in the literature a number of reports of the successful isolation and identification of AChR macromolecules from the electric organs of *Electrophorus electricus*, *Torpedo californica*, and *Torpedo marmorata* [76, 77, 78, 79, 80, 81].

The functional AChR was found to be a lipoprotein [78, 79], which may possess two pharmacologically distinct sites [80]. In its purified form the receptor protein was estimated to have a molecular weight in the range of 83000 to 112000 daltons [76, 83], although a somewhat higher value has also been reported [80]. It has been suggested that the AChR is a doughnut-shaped globular molecule [83].

In its binding of isotopically labelled ACh the purified receptor exhibits both positive cooperativity [84] (at low concentrations of ACh), as well as negative cooperativity [83] (at higher concentrations of ACh). There is some indication that the purified protein differs in its binding properties from both the solubilized as well as the membrane bound AChR [85].

The receptor macromolecule is highly acidic, having an isoelectric point of 4.8 in its purified form. However, since the protein has a considerable tendency to aggregate, it was suggested that there are areas of high densities of positive charge in the molecule, despite its overall negativity [83].

Although there are many similarities in the properties of the AChR and the enzyme AChE, the two proteins were found to be clearly separable [86, 87], and they do not seem to have a common subunit [76]. Nevertheless the amino acid composition of AChR and AChE is not very different. The basic amino acids make up approximately 12 mole % of either macromolecule, but AChR has about 33% more lysine and 33% less arginine. The acidic amino acids constitute about 20 - 23 mole % of either protein, with approximately equal representation by aspartic and glutamic acids. AChR has a higher mole % of threonine, half cystine, and isoleucine, and less of glycine and valine [83].

There is considerable evidence that disulfide bonds and exposed sulfhydryl groups may be of importance in receptor activity [78, 79]. They are apparently essential for preserving the appropriate conformation of the AChR, necessary for its binding of ACh. Similarly it has been suggested that arginine or lysine and tyrosine or phenylalanine may also be of importance for receptor functioning [83]. The relationship of these groups to the receptor active site has not yet been elucidated.

In the study of the electrical properties of artificial membranes containing a hydrophobic protein isolated from Electrophorus electricus, which was cited in the last section, the activation of a permeability system by ACh and certain of its analogues was investigated [75]. In each case a different rise time and decay of the transient response produced by these

agents was observed.

The concentration of the compounds required to generate a permeability change was found to be much larger than in the case of biological membranes. This has been attributed to the fact that the lipid matrix in which the receptor is embedded is different in the two types of membranes. It was observed that in the presence of uranyl ions the response to ACh was increased by a factor of a hundred [88]. In this context it may be noteworthy to point out that the artificial membranes did contain phosphatidylcholine.

Theoretical Aspects of Drug-Receptor Interactions

While there is some knowledge at present concerning the ionic processes involved in chemically induced membrane conductance changes, as well as of the nature of the chemical environment responsible for such changes, relatively little is known concerning the molecular mechanisms that underlie these changes. However, a large amount of indirect evidence as to the nature of these mechanisms is available in the form of pharmacological data. Studies of the transmitter substances, and drugs that mimic their action (agonists), or that block it (antagonists), have lead to some qualitative conclusions concerning the chemical substituents and structure a molecule must possess to induce a membrane conductance change resulting in an observed physiological response. This information can be used to draw conclusions about the

nature of the molecular interactions that are involved in the formation of the neurotransmitter-receptor complex.

Most commonly the pharmacological data consists of tabulations of some parameters expressing the magnitude of the response, or the affinity of the receptor for a drug, for a series of chemically similar compounds. Unfortunately due to factors such as the non-specific absorption of the drug, the measured physiological response may not bear a direct relationship to the formation of the drug receptor complex. Small differences in the experimental values are therefore of little significance.

Generally, the activity (or equivalently, the potency) of an agonist is expressed in terms of the number of moles of that agent required to produce the same response as a standard compound.

A quantity known as the pD_2 value is often used as a measure of the affinity of a receptor for a given agonist. (If no confusion about the nature of the receptor is possible, one may simply speak of the affinity of that agent). The pD_2 value is defined in terms of the agonist concentration required to produce 50 per cent of the maximum response, $[A]_{50}$, as

$$pD_2 = -\log\,[A]_{50} \quad .$$

If the drug-receptor interaction is assumed to be a simple absorption process (as discussed below) it may be shown that $[A]_{50}$ is inversely proportional to the equilibrium constant K_A for that process, i. e.

$$K_A = \frac{1}{[A]_{50}} .$$

One of the earliest theories put forward to explain drug action implied that the interaction of an agonist (A) with the receptor (R) is essentially a process of absorption that can be expressed as

$$A + R \underset{k_2}{\overset{k_1}{\rightleftarrows}} RA$$

and for which there exists an equilibrium constant K_A, where

$$K_A = \frac{k_1}{k_2} .$$

It was suggested that the rate of agonist-receptor complex (RA) formation, k_1, is a measure of the rate of the production of a response. The response would thus be proportional to the number of agonist-receptor interactions, and the maximum response would correspond to an occupation of all the available receptors. This theory was, however, found inadequate. It could not easily encompass the fact that there are some compounds, known as partial agonists, which, regardless of their concentration level, do not produce the same maximum response that is produced by agonists.

Consequently, a number of alternative theories were proposed which explicitly involved a parameter expressing the innate ability of a drug to produce a response [89, 90]. It was suggested that drug action at a receptor might resemble enzyme-substrate interactions. A quantity known as the intrinsic

activity was postulated to be proportional to the reaction rate constant k_3, which determines the formation of the final products in the hypothetical reaction

$$A + R \underset{k_2}{\overset{k_1}{\rightleftarrows}} RA \overset{k_3}{\rightarrow} \text{Response} + A + R \ .$$

Partial agonists were thought to be characterized by a relatively low k_3, and therefore a low intrinsic activity. However, even in this modified form of the initial theory, their action would not be adequately explained [91]. The postulated intervention of a rate-limiting change in an initially inert drug-receptor complex implies that there is a unique energy barrier for the formation of the transition state leading to the production of a response. The rate constant for that step can therefore not be used to distinguish between agonists and partial agonists.

The meaning of the intrinsic activity parameter was subsequently reinterpreted to reflect the fraction of collisions between a drug and the receptor that are effective in producing a response [92]. This statistical definition does not refer to a fixed mechanism for the processes taking place and is therefore more acceptable than the previously suggested one.

In spite of the controversies it has provoked the intrinsic activity is often used to express the ability of an agonist to initiate a response. Intrinsic activities are assigned by

comparing the maximum response of a given agonist to that produced by a standard compound.

According to another theory [93], which can be more effectively related to events at the molecular level, the rate of complex formation with receptors would determine the response, rather than it constituting an obligatory step preceding the activation phase. A high stimulant activity would thus reflect not only high rate of combination with receptors, but an even higher rate of dissociation of the drug-receptor complex. Thus agonists would be characterized by a high rate of dissociation k_2, partial agonists by an intermediate k_2 value, and antagonists by a small k_2 value. While the theory successfully accounts for the existence of partial agonists and antagonists, its application in a number of specific cases leads to rather implausible results [91].

The evident inability of theories, starting from general presuppositions, to consistently describe the molecular events involved in determining the potency of compounds, has led to the suggestion of a somewhat more specific theoretical framework [91]. It was proposed that in analogy to the interaction of an enzyme and its substrate, agonists are able to induce a conformational change in the receptor, which would lead to molecular changes resulting in a response. In addition to the interaction between a specific agonist A_S and the receptor

$$A_S + R \rightleftarrows R^*A_S$$

that results in an active receptor protein conformation R^*A_S, there may be nonspecific interactions (involving a nonspecific agonist A_n)

$$A_n + R \rightleftarrows R^{\dagger}A_n$$

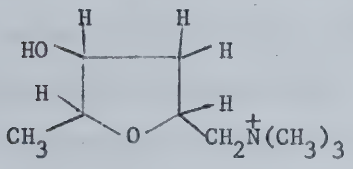
that result in an inactive conformation $R^{\dagger}A_n$. The degree to which molecules possess the necessary molecular features to induce an active rather than an inactive conformation would determine whether they are agonists, partial agonists, or antagonists. The distinguishing feature of this proposal is, of course, that binding at the receptor may either facilitate or hinder the generation of the response.

Pharmacological Data

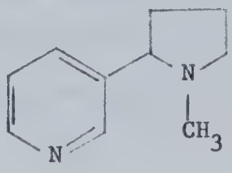
With the aim in mind of establishing the factors that contribute to potency, as well as those that diminish it, a review of some of the more pertinent pharmacological data, available for agonists and partial agonists, will now be given. Antagonists will not be considered, since their general mode of action is merely to block the access of the natural transmitter substance to the receptor site [94].

Pharmacological investigations have shown that cholinergic synapses within the ANS may be distinguished on the basis of the action of drugs at these synapses. In particular, there

is a similarity in the effects that ACh and muscarine produce at parasympathetic postganglionic synapses, while at low concentrations nicotine mimics the action of ACh at ganglionic synapses.



Muscarine



Nicotine

The division into muscarinic and nicotinic nerve tracts is, however, not absolute. There are many analogues of ACh, which like ACh itself, possess both nicotinic as well as muscarinic properties.

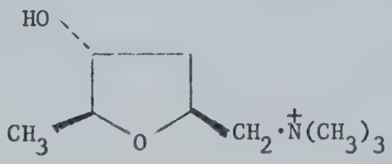
It is possible to classify the various cholinomimetic agents that have been investigated according to their muscarinic and nicotinic stimulant activities. The ordering of the compounds listed will reflect their ability to produce the same response as ACh at a given site and, as such, the information will be a generalization of the trends observed in a number of diverse tissue preparations. It is therefore necessary to point out that, while the relative ordering is almost always the same at the various receptor systems, the number of moles of a compound required to produce a standard response may vary considerably. A listing of agonists based on such a scheme results in four

broad classes of compounds: agents that are at least as potent as ACh at (1) muscarinic sites, (2) at both muscarinic as well as nicotinic sites, (3) at nicotinic sites, and (4) agents that are less potent than ACh at both sites. The last class should contain numerous derivatives of the compounds in the former classes, which are less potent than the parent compound. It will however be explicitely limited to prototypes of compounds structurally unrelated to those.

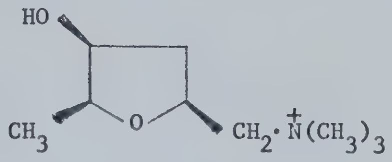
Further it may be noted that in speaking of the general features of agonists, it is often convenient to refer to certain key functional groups such as the ether oxygen of muscarine or the carbonyl oxygen of ACh - even in compounds that do not explicitly contain these but have chemically similar centers. In such cases the use of quotation marks will serve to indicate the unconventional usage of the terms.

(1) Predominantly Muscarinic Agents

a) L(+)-muscarine is the most stereospecific, though not the most potent, muscarinic agent. Of the four pairs of enantiomers only the compound having the following general structure (muscarine)



is equiactive with ACh as a muscarinic stimulant [95]. The compound epimuscarine

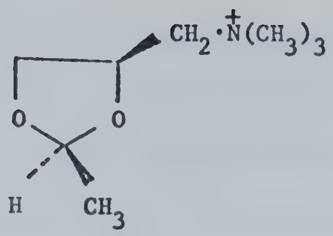


is about a hundredth as active, while the optical isomer D(-)-muscarine is over three hundredth as active [95].

As will be observed with all close ACh analogues, the effect of removing the terminal methyl group of muscarine is to reduce the activity. However, the corresponding demethyl compound for epimuscarine is almost as active as DL-epimuscarine itself [95]. The 2-methylated analogue of muscarine is even less active at muscarinic sites than the demethyl compound [95]. In contrast to the large difference in potency between DL-muscarine and DL-epimuscarine, both DL-4,5-dehydromuscarine and DL-4,5-dehydroepimuscarine are approximately equipotent to DL-muscarine [96].

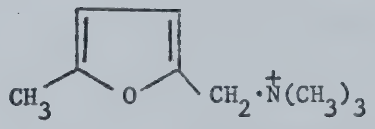
Muscarine and its close analogues all exhibit a low nicotinic activity.

b) Another compound, that is as potent a muscarinic agent as ACh but has considerably less nicotinic activity, is L-cis-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide [97] (methyldilvasene)



The corresponding trans isomer is only slightly less active [97], indicating that, unlike in the case of the muscarines, the relative orientation of the 2-methyl and 5-quaternary groups is not highly critical. However, the removal of the 2-methyl group entails an approximately thousand fold loss in activity, and the addition of a second methyl group in the 2 position likewise leads to a decrease in activity [97].

c) 2-Methyl-5-dimethylaminomethylfuran methiodide (methylfurtrethonium) is yet another compound that is approximately equipotent to ACh [98] as a muscarinic agent but possesses a very low nicotinic activity



The effect of replacing the 2-methyl group by hydrogen is to reduce the activity by only a factor of ten, while the 2-ethyl compound is less than a hundredth as active [98]. The corresponding saturated compound 2-methyl-5-dimethylaminomethyltetrahydrofuran methiodide is also less than a tenth as active as the furan compound [98].

Clearly all the agents in this class contain an oxygen structurally equivalent to the ACh "ether" oxygen, in addition to the cationic head group. Furthermore, optimum activity is achieved only if there is a terminal methyl group at a distance corresponding to the length of an extended five-atom chain from the cationic group. The presence of a larger alkyl group at that position leads to a decrease in activity. Methylfuretrethonium is conspicuous in that it does not contain an oxygen structurally corresponding to the ACh carbonyl oxygen, as do muscarine and methyldilvasene.

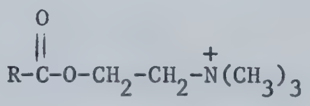
It is noteworthy that while these compounds have relatively low nicotinic activities, the affinity of the nicotinic receptor for each agent is about the same as that of the muscarinic receptor [99,100]. Muscarine is about half as potent a nicotinic agent as are the other two representatives of this class. The corresponding demethyl compounds all have a lower affinity but a higher activity than do the parent compounds.

(2) Agents that are both Muscarinic and Nicotinic Agonists

a) Acetylcholine, which is generally used as the reference compound for establishing the activity of other agents, is, by definition, a potent agonist. However, while ACh is certainly among the most potent muscarinic agents, there are a number of nicotinic agonists which are considerably more potent. This has led to speculations that ACh may not be the prototype for a nicotinic agent, and that its high natural nicotinic activity

may merely be attributable to the fact that it is released in such large quantities at a location very near the receptor site [101]. As in the case of the compounds of the previous class, the affinities of the nicotinic site and the muscarinic site for ACh are about the same [99, 100].

The structure-activity relationships for the muscarinic actions of choline esters

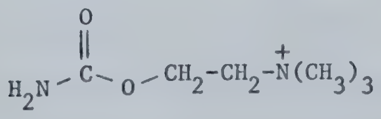


$$R-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3$$

reconfirm the five-atom rule that was proposed for muscarinic agents. ACh is clearly the most potent member of the series. While the formyl ester is considerably less active there is also a steady decrease in activity with an increasing size of the R-group, beyond that of a methyl group [102]. Aromatic esters are virtually inactive as muscarinic agents [103].

In contrast, there is some evidence that the optimum nicotinic activity is actually reached with the ethyl ester [104]. Furthermore, a high potency is also exhibited by aromatic esters - particularly those with the aromatic group at the distance of a two-carbon chain from the carbonyl carbon [103].

Carbaminoylcholine is both a potent muscarinic

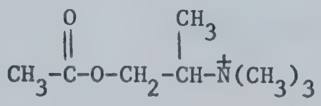


$$H_2N-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3$$

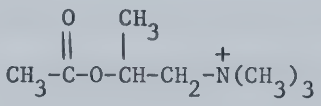
and nicotinic agent [99, 105]. Its intrinsic activity at nicotinic

sites was reported to be twice that of ACh.

The stereospecificity that the AChR exhibits in its interactions with acetyl-α-methylcholine and acetyl-β-methylcholine [106] provides some insight into the possible structural arrangement of the binding *loci* at the receptor site.



$$CH_3-\overset{\overset{O}{\|}}{C}-O-CH_2-\overset{\overset{CH_3}{|}}{CH}-\overset{+}{N}(CH_3)_3$$



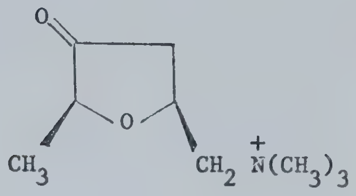
$$CH_3-\overset{\overset{O}{\|}}{C}-O-\overset{\overset{CH_3}{|}}{CH}-CH_2-\overset{+}{N}(CH_3)_3$$

Of the two optical isomers of acetyl-α-methylcholine, the D(+)-compound is almost a hundred times more active at muscarinic sites than the L(-)-compound. Nevertheless its activity is only about one thirtieth that of ACh. In contrast, the nicotinic site shows very little stereospecificity, the activity of DL-acetyl-α-methylcholine being about one half that of ACh.

L(+)-acetyl-β-methylcholine is equiactive with ACh, and is over two hundred times more active than the D(+)-isomer as a muscarinic agent. The nicotinic activity of DL-acetyl-β-methylcholine is almost two hundred times less than that of ACh, and the D(+)-isomer is reported to be the more active compound.

b) While the configurational specificity of the AChR towards muscarine and its analogues is quite high, receptor

interactions with muscarone



are relatively non-specific [96].

In contrast to the compounds of the previous class, it is the D(-)-isomer of muscarone that is most active at both muscarinic and nicotinic sites. However, the difference in activity between the two optical isomers is not very pronounced.

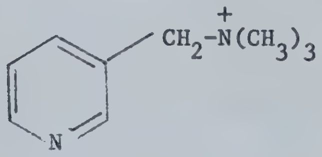
Quite in accordance with the five atom rule, the 2-demethyl compound as well as the 2-methylated compound are less active than muscarone by a factor of ten, at muscarinic sites. DL-4,5-dehydromuscarone is almost equipotent with ACh.

Aside from the fact that ACh is not a cyclic compound, the main feature that serves to distinguish the representatives of this class from those of the previous one is the presence of a carbonyl group. No doubt the marked nicotinic activity of ACh and muscarone can be attributed to some property of that functional group. The data for choline esters seem to suggest that the requirements for stimulant activity are much more strict at muscarinic sites than at nicotinic sites.

(3) Predominantely Nicotinic Agents

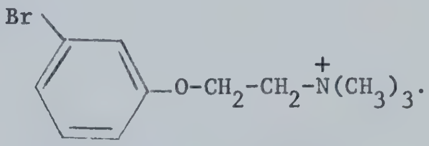
a) Nicotine and certain of its analogues are among the most potent nicotinic agents known [107], exhibiting a ganglionic

stimulant activity ten times larger than that of ACh. 3-N,N-dimethylaminomethylpyridine methiodide



is a particularly potent nicotine analogue, being twice as active as nicotine.

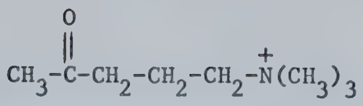
b) Choline phenyl ether and substituted choline phenyl ethers are even more active nicotinic agents than nicotine [107]. In particular, the meta-bromo compound was reported to be forty times more potent than nicotine



At muscarinic sites the aromatic choline ethers are practically devoid of activity [108] while nicotine and its analogues are actually antagonists [109].

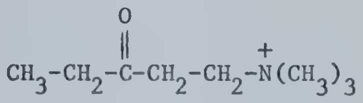
c) A number of ketoalkyltrimethylammonium compounds [110] are approximately equipotent with ACh as nicotinic agents. Their muscarinic activity was found to be much less pronounced.

The 4-ketoamyltrimethylammonium ion is the most

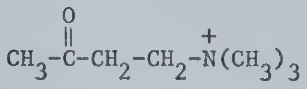


$$CH_3-\overset{\overset{O}{\|}}{C}-CH_2-CH_2-CH_2-\overset{+}{N}(CH_3)_3$$

active muscarinic agent, while the 3-ketoamyltrimethylammonium ion and the 3-keto-butyltrimethylammonium



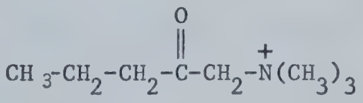
$$CH_3-CH_2-\overset{\overset{O}{\|}}{C}-CH_2-CH_2-\overset{+}{N}(CH_3)_3$$



$$CH_3-\overset{\overset{O}{\|}}{C}-CH_2-CH_2-\overset{+}{N}(CH_3)_3$$

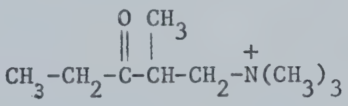
ion are considerably less active at most muscarinic preparations.

It is interesting to note in this context that neither the 2-ketoamyltrimethylammonium ion nor



$$CH_3-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-CH_2-\overset{+}{N}(CH_3)_3$$

the 3-keto-2-amyltrimethylammonium ion are



$$CH_3-CH_2-\overset{\overset{O}{\|}}{C}-\overset{\overset{CH_3}{|}}{CH}-CH_2-\overset{+}{N}(CH_3)_3$$

very potent at muscarinic and nicotinic sites.

Although a number of other compounds such as certain n-alkyltrimethylammonium ions and hydroxyalkyltrimethylammonium ions were reported to be as active or more active nicotinic stimulants than ACh at specific preparations, insufficient data are available to warrant the conclusion that these agents are generally potent nicotinic agonists. They will therefore be classed among the next series of cholinomimetic agents.

An investigation of the preceding data seems to suggest that the requirements for nicotinic activity are not very strict. While the high potency of the keto-compounds again seems to implicate a carbonyl functional group as an activating feature, which could have a structural and functional analogue in the pyridine nitrogen of nicotine-like compounds, such a feature is clearly lacking in the case of the aromatic ethers. This anomaly becomes even more pronounced in view of the observed activities of the members of the aliphatic choline ether series which will be discussed in the next section. Finally, it should be noted that the five atom rule does not appear to hold for nicotinic agents, since the 3-ketobutyltrimethylammonium compound is about twice as active a nicotinic stimulant as the 3-ketoamyltrimethylammonium ion.

(4) Weak Muscarinic and/or Nicotinic Agents.

a) Of the aliphatic choline ethers [110] the ethyl compound

$$CH_3-CH_2-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3$$

is the most potent muscarinic agent, being one tenth as active as ACh at most preparations. Methyl homocholine

$$CH_3-O-CH_2-CH_2-CH_2-\overset{+}{N}(CH_3)_3$$

ether and n-propyl formocholine ether are over one fortieth

$$CH_3-CH_2-CH_2-O-CH_2-\overset{+}{N}(CH_3)_3$$

as active as ACh as muscarinic agents.

The situation is somewhat reversed at nicotinic sites where the latter two compounds are almost twice as active as ethyl choline ether. Vinyl choline ether was also found to be more potent than the ethyl ether [111].

b) Alkyltrimethylammonium compounds are generally much less active muscarinic agents than ACh; however in certain specific preparations unusually high potencies have been reported for these compounds [112]. The n-amyl compound is by far the most potent representative of the saturated alkyltrimethylammonium series, its activity at most muscarinic preparations being slightly less than that of the methylhomocholine ether.

Due to the scantness of the available data it is difficult to make a general statement concerning the muscarinic activities of the unsaturated alkyltrimethylammonium compounds. However, both the doubly bonded as well as the triply bonded representatives have a sequence of potencies in agreement with the five atom rule [113]. For the compounds having the general structure

$$\mathrm{R\text{-}CH{=}CH\text{-}CH_2\text{-}\overset{+}{N}(CH_3)_3}$$

the trans isomer appears to be more active than the cis isomer, while for the compounds with the general structure

$$\mathrm{R\text{-}CH{=}CH\text{-}CH_2\text{-}CH_2\text{-}\overset{+}{N}(CH_3)_3}$$

both isomers have the same activity. The strongest muscarinic activity for the unsaturated agents was reported for the compounds

$$CH_3\text{-}CH_2\text{-}C{\equiv}C\text{-}CH_2\text{-}\overset{+}{N}(CH_3)_3$$

and

$$CH_3\text{-}C{\equiv}C\text{-}CH_2\text{-}CH_2\text{-}\overset{+}{N}(CH_3)_3$$

In contrast to these alkyl agents, aryl and aralkyl compounds are relatively inactive as muscarinic stimulants [114].

The optimum nicotinic activity for the series of saturated alkyl compounds is reached with the butyltrimethylammonium ion which is slightly more active than the amyltrimethylammonium ion. The difference in the potencies of these agents is not attributable to a difference in their affinities. The effect of introducing a double or a triple bond into the alkyl chain is to increase the activity slightly over that of the saturated analogue. The compound

$$CH_2{=}CH\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\overset{+}{N}(CH_3)_3$$

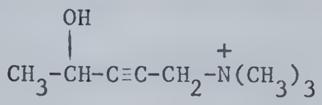
was reported to be particularly active [101].

c) Hydroxy-alkyltrimethylammonium compounds such as choline are all relatively inactive as muscarinic agents [113]. The optimum muscarinic activity was reported for the compound

$$HO\text{-}CH_2\text{-}CH_2\text{-}C{\equiv}C\text{-}CH_2\text{-}\overset{+}{N}(CH_3)_3.$$

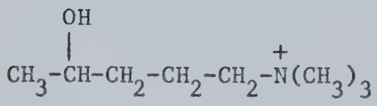
The presence of the hydroxy group also does not appear to

enhance the activity of these compounds as nicotinic agents compared to the alkyl series. The compounds



$$CH_3\text{-}\overset{OH}{CH}\text{-}C{\equiv}C\text{-}CH_2\text{-}\overset{+}{N}(CH_3)_3$$

and



$$CH_3\text{-}\overset{OH}{CH}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\overset{+}{N}(CH_3)_3$$

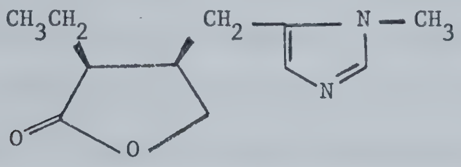
were reported to be particularly potent representatives.

In summary it may be stated that the low activity of these agents may be due to the absence of some of the characteristic features of ACh and its closer analogues. The member most active at muscarinic sites in the ether series is indeed the compound whose ether oxygen is structurally more similar to the corresponding ether oxygen of ACh. Surprisingly, however, this is also the least active nicotinic agent. This would indicate that the presence of an ether oxygen tends to enhance muscarinic activity while it actually diminishes nicotinic activity.

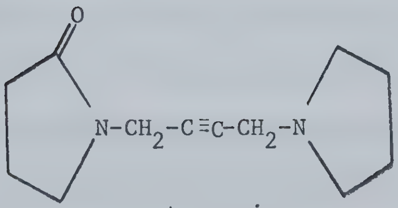
In the case of the alkyl series the ability to conform to the five atom rule seems to be of prime importance in determining muscarinic and, to a lesser extent, also nicotinic activity. The presence of an unsaturated region also appears to enhance the nicotinic potency of these compounds, as well as those in the ether series.

(5) Miscellaneous Agents

A number of potent muscarinic agents possessing structural features differing from those of the close analogues of ACh, have been reported. The most noteworthy examples are [100, 113]

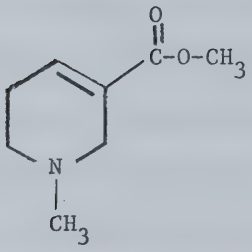


pilocarpine



oxotremorine

and



arecoline

IV. A MODEL FOR CHOLINERGIC RECEPTORS

Introduction

In Chapter III evidence was presented for the fact that the AChR is a macromolecule with chemoreceptive properties for a very specific class of compounds. The approach of an agonist causes a perturbation of the state of the receptor, as well as perhaps its immediate membrane environment, which initiates a chain of molecular events culminating in a physiological response. Presumably there is a local region at the receptor that has the genetically endowed disposition of being able to interact specifically with the class of compounds that qualify as agonists. In analogy to enzyme systems, this may be termed the active site of the receptor macromolecule. It has already been noted that there are two functionally different AChR's, which may be distinguished on the basis of the effects produced by muscarinic and nicotinic agents. The question can be raised as to whether this functional difference is ultimately attributable to a difference in the chemical substituents of the respective active sites of the two types of receptor, or whether it is due to a different structural arrangement of the same substituents. In extension a similar question applies to what is presumably the same type of receptor in different tissue preparations, where the amount of a given agonist required to produce a standard response can vary considerably. The explicit investigation of

this point will be deferred until some pertinent facts have been discussed, and, unless stated otherwise, the term "AChR" will be rather indiscriminately used to apply to any receptor at which ACh is an agonist.

In Chapter III a preliminary attempt was made to identify the molecular features common to pharmacologically active agents of a given class. On the basis of that investigation, as well as the information available on suitable enzyme systems, a model for the active site of the AChR will be presented in this Chapter. A more detailed study of the structure-activity relationships of agonists and partial agonists will then be undertaken to refine the model. Further an attempt will be made to rationalize the difference between muscarinic and nicotinic action.

Receptor Models

Before proceeding with this scheme a review of some of the many models that have been proposed is in order. Due to the present lack of knowledge concerning the detailed architecture of the receptor active site, most models have been limited to sketching the rough compartments sterically available to agonists, and to depicting the approximate positions of local positively and negatively charged regions. Since the observed structure-activity relationship for agonists is stricter at muscarinic sites than at nicotinic sites, models for the muscarinic receptor are particularly numerous.

One such diagrammatic representation of the muscarinic receptor was given by Beckett [115]. The model entails an anionic cavity to accommodate a quaternary nitrogen, a positively charged group about 3 Å away to accommodate the ether linkage of muscarine or the ester linkage of ACh and its analogues, and another positively charged area, about 5 - 7 Å away, to accommodate the hydroxyl group of muscarine, the carbonyl oxygen of ACh and its analogues, or the conjugated ring of the furan analogues of muscarine. Further that author suggests that there is a region of accessory binding for antagonists, at an even larger distance from the anionic group.

According to another model for the muscarinic receptor, originally put forth by Belleau [116] and modified by Friedman [113] the agonist binding site consists of two distinctly different areas. There is a small negatively charged cavity, and a larger, less enclosed compartment. Due to the limited size of the anionic site optimum binding of the agonist cationic groups would not occur. However small organic cations, if they approach closely enough, might induce a stronger interaction that would initiate a muscarinic response.

The sole function of the groups at the larger secondary region is to orient the agonist molecule so that the cationic head group can optimally bind at the anionic site. Two attractive forces acting on the "ether" oxygen and the terminal methyl group, and a repulsive force acting on the carbonyl

oxygen of ACh, or a structurally equivalent group, are suggested to be involved in this process.

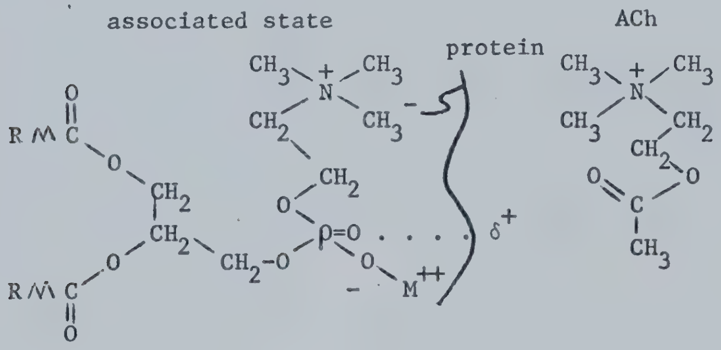
In contrast to these previous models, where the emphasis has been on electrostatic interactions between the agonist and the receptor, Schueler proposed that the ability of the agonist molecule to polarize the receptor substituents may determine its activity [117]. On the basis of some calculations he postulated that for any series of related groups on the agonist the potency decreases as the absolute value of the polarizing force varies from that for ACh.

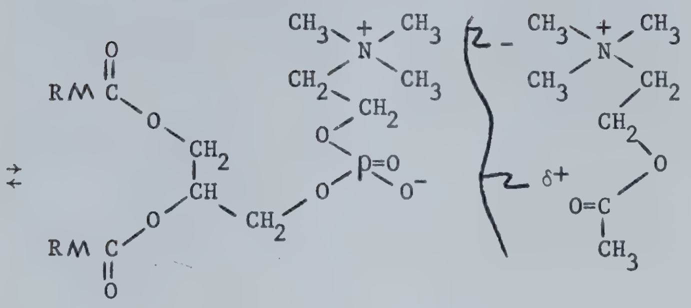
As was already indicated the task of finding a model that can explain the binding of agonists at nicotinic sites is not easy. Hey's proposal [108] that optimum activity is associated with compounds that can adopt the polarized form

$$\overset{\delta-\,\delta+}{R-O}-CH_2-CH_2-\overset{+}{N}(CH_3)_3$$

would suggest that the complementary binding region at the AChR is negatively charged. On the other hand Kier has concluded from his EHT calculations of nicotine [118] that the binding region corresponding to the pyridine nitrogen of nicotine should be positively charged. This latter proposal is supported by the finding of Wong and Long [119] that in phenacyltrimethylammonium compounds the nicotinic potency decreases as the electron density around the carbonyl oxygen decreases.

It should not be forgotten that ultimately the static receptor substituents that are the distinguishing features of these models are actively involved in conjunction with an agonist, and perhaps a membrane constituent, in bringing about a series of ionic processes. Recent experimental findings indeed suggest that phospholipids may take part in such a mechanism. Therefore a proposal, put forth by Watkins to explain the mode of action of agonists [50] and extended by Ehrenpreis to include antagonists [120], may be particularly pertinent. In this formulation account is taken of the fact that the AChR is an integral part of the synaptic membrane, and that the membrane lipid phosphatidylcholine has a polar group resembling ACh. Attachment of ACh and other agonists to the AChR is thought to disrupt the protein-lipid binding, which somehow leads to a change in the conductance to ions.




dissociated state

The phosphate group of phosphatidyl choline is believed to be linked to the protein by two points - the phosphoryl-oxygen and via a metal ion which separates in the binding process. Ehrenpreis has further suggested that the role of antagonists may be to stabilize the protein-lipid complex against dissociation by ACh.

Another proposal that may be of relevance for a mechanistic treatment was made by Belleau [91], as a result of his extensive studies of the binding of compounds at the AChE active site. In analogy to the case of AChE, the ability of agonists to displace strategically bound water molecules on the AChR, which are thought to be required for the transport of ions through pores in the membrane, is suggested by that author to be an explanation of their activity.

This brief survey will have indicated that there is no general agreement about the modes of binding of agonist molecules at the AChR active site. The opinions of various authors diverge rather sharply over the nature of the interaction between the

carbonyl group of ACh (or structurally equivalent nucleophilic groups on other agonists) and a complementary site at the receptor. Beckett implies that a binding interaction with a positively charged receptor region takes place, while Belleau postulates the presence of a nucleophilic group resulting in a repulsion of the agonist. In order to explain the pronounced nicotinic activity of the compounds of class two (according to the scheme of Chapter III), the possibility of covalent bonding between the carbonyl carbon of these agents and a basic group at the receptor has also been invoked [95]. Although the details of this binding process have remained unelaborated, a similar interaction can evidently not take place with such potent nicotinic agents as the choline phenyl ethers and the nicotine analogues. The subsequent need to postulate multiple binding sites to explain the action of agonists at nicotinic receptors must be considered a weakness of this proposal.

On the basis of a number of general considerations derived from the study of enzyme-substrate and enzyme-inhibitor relationships, Triggle [100] has suggested that there exist at least two distinct modes for the binding of cholinomimetic agents at the AChR. One receptor region is thought to be responsible for the binding of polar agents, while at an adjacent region, sharing the same anionic binding group, nonpolar molecules are believed to be preferentially bound. The most direct evidence put forth in favor of this proposal rests on the trends in the difference

between the free energy of binding of an agent and the free energy of binding of the corresponding compound lacking the terminal methyl group, for a series of polar and nonpolar compounds conforming to the five atom rule [121]. The order of the incremental change in free energy for the binding of a methyl group was reported to be

ACh ~ methyldilvasene >> methylfurtrethonium > propylcholine ether
~ nicotine >> n-amyltrimethylammonium ion.

The fact that there is a comparatively large difference in the value of this quantity for polar and nonpolar agents was cited as evidence for the overall difference in the binding mode of these compounds.

If this argument were correct it would be necessary to reclassify the agents considered in Chapter III according to their polar character, and two distinct binding sites would have to be investigated. It seems, however, that the reported trend in the incremental change in free energy can be explained in a different fashion. There exists strong evidence that water at the surface of a protein is much more structured than bulk phase water [122, 123]. This suggests that as a molecule approaches the receptor, it will be separated from the solvent layer that surrounds it in the bulk solution. The enthalpy change for the desolvation step will be largely determined by the number of hydrogen bonding sites a molecule possesses. One can propose

that the effect of introducing a methyl group next to one of these polar sites will be to partly shield the site from the bulk solution, which would lead to a lower enthalpy change of desolvation. For the compounds used in the above study, this shielding effect would be expected to be particularly pronounced in the case of ACh and methyldilvasene, since in these agents the methyl group is next to two polar sites. It therefore seems that the reported trend in the incremental change in free energy change may largely reflect the difference in the enthalpy change of desolvation rather than a difference in the enthalpy of binding at the receptor. Nevertheless a solvation effect cannot be responsible for the relatively big difference in values for propylcholine ether and the n-amyltrimethylammonium ion. A separate binding mode at the AChR for the latter compound therefore does not seem out of the question.

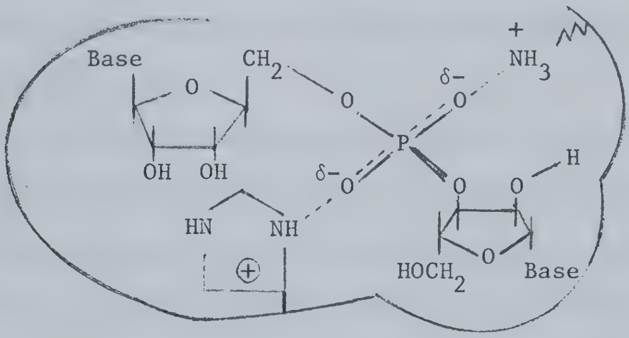
Proposal of a Model for the AChR Active Site

The preceding examples suggest that due to a lack of information concerning the receptor active site there is a certain amount of freedom in the variety of models that can be proposed. The critical role that calcium ions and possibly also lipids play in the production of a membrane conductance change indicates the need to take into account factors other than just the optimum binding of potent agonists in designing receptor models.

The recent finding that the AChR isolated from certain tissues is partly proteinic in nature, gives some support to the frequently encountered proposition that agonist-receptor interactions resemble enzyme-substrate interactions. It must be born in mind, however, that in contrast to the action of enzymes, the AChR presumably does not promote a making or breaking of covalent bonds in the agonist. This possible similarity in action suggests that, in order to investigate what binding groups may be present at the AChR active site, it might be useful to study enzyme systems that can specifically bind ACh-like substrates. Furthermore since the involvement of lipids seems probable, the focus will also be on enzymes that can accommodate the polar portions of lipids in their active site - in particular the phosphate group of phosphatidylcholine.

AChE is an enzyme that possesses these properties. Not only are ACh and some of its esteric analogues substrates of the enzyme, while the nonesteric analogues act as inhibitors, but there are also a number of phosphate and phosphorothiolate compounds that are potent competitive inhibitors [124]. Of the amino acid residues that constitute the active site only two are not directly involved in the enzymatic activity, and thus serve solely to bind the substrate specifically [44]. This is the anionic residue, presumed to be the α-carboxyl of glutamic acid, and an unknown acidic group of pk 9.2, which probably acts as a proton donor to the ether oxygen of ACh and its analogues, as well as to the corresponding oxygen in the phosphate group of inhibitors.

Another example that might be pertinent in this context is bovine pancreatic ribonuclease, which is one of the best defined enzymes in terms of both structure and function [125]. The enzyme hydrolyzes RNA in the intestine, to produce 3'-nucleotides. The initial binding of the substrate, depicted below, involves the fixation of a phosphate linkage by means of a histidine and a lysine residue.



Clearly histidine acts as a proton donor, while the lysine chain interacts electrostatically with the relatively delocalized formal negative charge on the phosphate group. The point of interest is that the polar group of phosphatidylcholine could be similarly bound at the AChR, as was suggested by Watkins. If ACh indeed displaces the lipid, its carbonyl oxygen could be attached to either one of the two binding groups.

As was already indicated, the presence of a terminal methyl group on cholinergic agonists is a factor that influences the activity of these compounds at muscarinic sites and, to a lesser extent, also at nicotinic sites. The three serine proteases - chymotrypsin, trypsin and elastase - offer evidence that the specific

binding, and steric fit, of substrate portions immediately adjacent to the moiety acted upon by the enzyme can be of decisive importance [125]. Chymotrypsin breaks only those peptide bonds adjacent to large hydrophobic side chains, trypsin cuts peptide bonds adjacent to positively charged side chains, while elastase attacks bonds that lie next to very small side chains. In each case the specificity of action is determined by the ability of a section of the substrate to occupy a complementary region at the enzyme.

These examples provide some information concerning the nature of the amino acid residues that may constitute the AChR active site. It is possible to make a preliminary specification of the types of interactions that would take place between agonist molecules and four hypothetical receptor bonding groups. Each receptor substituent will be named according to its complementary binding site on a prototype of the cholinomimetic agents, i. e. the quaternary ammonium group, the "ether" oxygen, the "carbonyl" oxygen, and the terminal methyl group. The type of interaction suggested to be involved will be chosen to reflect the largest component energy of the total energy of binding.

The following modes of binding may be postulated:

1) A predominantly electrostatic interaction between the quaternary ammonium group and an anionic site - possibly the carboxylate group of the conjugate base of glutamic acid.

2) Hydrogen bonding between the "ether" oxygen and a proton donor group.

3) Hydrogen bonding or an electrostatic interaction between the "carbonyl" oxygen and either a proton donor species - possibly histidine - or a positively charged amino acid residue - perhaps lysine.

4) The steric fit and/or Van der Waals binding of a methyl or other small alkyl group at a distance of approximately the length of an extended five-atom chain from the cationic head group.

The explicit specification of the amino acid residues, that may constitute the receptor active site, is at this point only tentative, and a more detailed discussion will be given in the next Chapter. Further it will be noted that no attempt has been made to distinguish between the muscarinic and nicotinic active site. The model outlined above is complementary in its binding characteristics to ACh which is the natural transmitter substance at both types of AChR.

Relation of Binding to Activity

It now remains to investigate to what extent the ability of agents to be bound at any of the sites that have been postulated reflects upon their activity. As was shown in Chapter III, the affinity is generally expressed in terms of the pD_2 value, which is believed to be proportional to the logarithm of

the equilibrium constant for the receptor-agonist interaction. As such the quantity is also related to the total free energy change of binding. It was already mentioned that the energy of desolvation constitutes part of the free energy change of binding, and that consequently caution must be exercised in using affinity data to discuss the binding of agents that differ in their overall polar character. Nevertheless there exists ample evidence, for a number of sufficiently similar compounds, that differences in activity cannot always be attributed to differences in the energy of binding at the receptor. Thus it was found, for example, that the affinity of the muscarinic and nicotinic receptors for the compounds of class one (and for ACh) is about the same. However, while all these agents are equipotent as muscarinic stimulants, ACh is a more potent nicotinic agonist than the compounds of the first class.

The most probable explanation for this lack of correlation between the total binding energy and the activity of certain compounds is that high potency is in part determined by the ability of agents to affect the rate of the rate-determining step in the production of a response. The magnitude of the response is presumably proportional to the number and the average life-time of the ionic channels that are opened. Thus activity may be associated with the ability of compounds to either lower the energy of activation for the opening of a pore, or to raise the energy of activation for the closing of a pore.

It would seem that an obvious objection to this proposal, based on the principle of microscopic reversibility, is that any change in the energy of activation would equally affect the rate of the forward and the reverse reactions. The lifetime of an open pore would thus after all mainly be related to the thermodynamic stability of the agonist-receptor complex, i. e., the energy of binding. There is a reason, however, why this objection may not be a valid one.

It has already been mentioned that the action of the sodium pump leads to a larger concentration of sodium ions in the external solution than inside the neurone. An opening of pores in the membrane would lead, at least locally, to the establishment of an ionic equilibrium, and, at the same time, to a change in the chemical environment of the receptor. It is conceivable that, as a result of this process, a new reaction pathway for the closing of a pore, differing from the one required by the principle of microscopic reversibility, may become available.

One may speculate that these molecular events could constitute a control mechanism for synaptic transmission. The initial transmitter-receptor interaction would lead to the formation of a thermodynamically relatively stable complex - a receptor/membrane configuration required for the maintainance of a pore. In order to assure the closing of the pores and the subsequent regeneration of a concentration gradient upon the completion of the ion exchange processes, the altered chemical surroundings of

the receptor could act as a catalyst for the displacement of the transmitter. Finally the hydrolysis of the transmitter substance by AChE would terminate the sequence of steps involved in the synaptic transmission.

On the basis of this proposal it may be suggested that the main factors responsible for the activity of agents are:

1) The presence of molecular features that lead to strong binding at the receptor active site

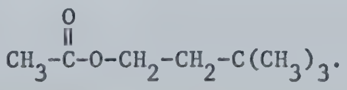
2) The presence of molecular features that lead to a kinetically stable agonist-receptor complex in the equilibrium-state ionic environment.

Agonist Binding at the Model Receptor Site

In order to determine the specific nature of these potency promoting factors, an investigation of the modes of binding of agonists at the receptor active site postulated above will now be undertaken.

(1) Binding at the Anionic Site

The presence of a cationic head group appears to represent the minimum requirement for a compound to be active at the AChR. This is evidenced by the fact that ACh is about three thousand times more potent than its uncharged isostere 3,3-dimethylbutyl acetate



$$CH_3-\overset{\overset{\displaystyle O}{\|}}{C}-O-CH_2-CH_2-C(CH_3)_3.$$

As a survey of the more active cholinomimetic agents shows, the cationic group is most commonly a quaternary ammonium function. However in a number of compounds, such as nicotine, arecoline, oxotremorine and pilocarpine, a tertiary ammonium function may be present instead.

The stepwise replacement of the methyl substituents in the quaternary ammonium group of ACh by either hydrogens or ethyl substituents leads to a steady and rapid decrease in activity at both nicotinic and muscarinic sites. One possible explanation for these trends is that any change in the cationic group will lead to a corresponding change in the binding distance to the receptor anionic group, which would also entail a less than optimum binding for the rest of the agonist molecule. Further it has been suggested that the ability to hydrophobically interact with a nonpolar region, in which the receptor anionic site is thought to be embedded, may be optimal for the agents containing a trimethylammonium function.

(2) Binding at the "Ether" Oxygen Proton Donor Site

With a few notable exceptions, all potent muscarinic agents contain a center that can act as a proton acceptor at the distance of approximately the length of a three atom chain from the quaternary nitrogen. Most commonly, as in all compounds of the first and second classes, this is an oxygen atom.

In certain cases the nucleophilic site is at a larger distance from the quaternary nitrogen. However it is noteworthy that

all these agents are tertiary ammonium compounds. Perhaps a shift due to the closer binding of the cationic group serves to bring the proton acceptor center of these compounds nearer to the receptor hydrogen bonding site.

The relative trend in potencies for the aliphatic choline ether series provides indirect evidence that hydrogen bonding is involved. It will be recalled that ethyl choline ether is about twice as potent a muscarinic agent as the methyl homocholine and the n-propyl formocholine compounds. Assuming optimum orientation, the distance of the oxygen atoms in the latter two cases to a receptor group facing the ethyl choline ether oxygen, would not be sufficiently large to cause a significant change in electrostatic or polarization interactions. This notable orientation and distance dependence of the interaction seems instead to be due to either covalent bonding or hydrogen bonding. Since ethers are known to be relatively unreactive, only the latter possibility appears to be likely.

There exists considerable evidence that binding at the "ether oxygen" site greatly promotes muscarinic activity. Thus while methyl homocholine ether and n-propyl formocholine ether are only slightly more potent than n-amyltrimethylammonium ion, all the keto-trimethylammonium compounds are actually less potent than that agent. Further it will be recalled that D(+)-acetyl-α-methylcholine was found to be a hundred times more active a

muscarinic stimulant than the L(-)-isomer. If the ability to interact with receptor binding sites complementary to L(+)-muscarine is assumed to be a criterion for potency, molecular models clearly show that the α-methyl group of L(-)-acetyl-α-methylcholine would greatly interfere with the binding of the "ether" oxygen of that compound. On the other hand, the α-methyl group of the D(+)-isomer would appear to have much less of a nuisance capacity. The fact that that agent is nevertheless not as potent as ACh may be attributable to a conformational constraint due to the presence of the α-methyl group, and perhaps to the steric hindrance that that group experiences at the receptor.

In contrast to the case of the muscarinic agents, it seems difficult to establish any correlation between the ability to bind at an "ether oxygen" site and nicotinic activity. It has already been noted that for the aliphatic choline ethers the possibility of increased binding tends to be associated with a loss in activity. On the other hand, the compounds of the first and the second classes, which differ in their nicotinic activities, all possess the proton acceptor group required for hydrogen bonding. Finally, the aromatic choline ethers are the most potent nicotinic agents known. In this context it may be pertinent to note that, while ethyl choline ether is relatively inactive and vinyl choline ether is somewhat active, choline phenyl ether is very active. It is questionable that this trend can be attributed to solely a difference in the strength of the bond formed with

the ether oxygen. Perhaps the observed increase in potency is due to the presence of a feature that promotes the stabilization of the agonist-receptor complex in the latter two compounds. The fact that both the keto-alkyltrimethylammonium agents, which lack an ether oxygen, as well as DL-acetyl-α-methylcholine are almost as potent as ACh, further suggests that the ability to bind at the ether oxygen site is not essential for nicotinic activity. The same holds, of course, for nicotine and its analogues which are actually more potent than ACh, but do not possess an ether oxygen, or a structurally equivalent proton acceptor group.

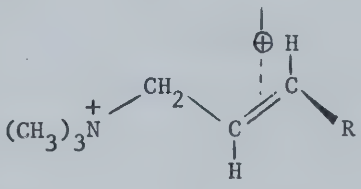
3) Interactions with the "Carbonyl Oxygen" Site

As was already indicated there is some question whether the receptor group, that specifically interacts with agonist functions corresponding to the ACh carbonyl oxygen, is a proton donor group or a positively charged amino acid residue.

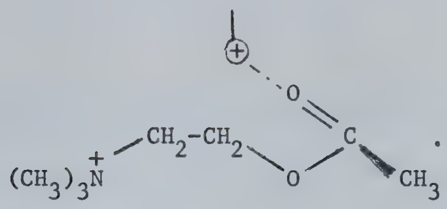
The high activity of methylfurtrethonium, which does not possess a proton acceptor center other than the ring "ether" oxygen, may be taken as evidence that muscarinic agents do not form a hydrogen bond with the receptor "carbonyl oxygen" site. This is further born out by the fact that the structurally similar tetrahydrofuran compound is ten times less potent. It seems that the larger potency of methylfurtrethonium cannot therefore be attributed solely to the presence of some structural feature leading to a stable agonist-receptor complex, but that a

specific interaction, which the furan ring may participate in is involved. In particular the attraction between the ring π-electron cloud and a positively charged receptor group may be implicated.

Further evidence that a positively charged group is present at muscarinic sites is provided by the structure-activity relationship for unsaturated alkyltrimethylammonium agents. It was noted that the 2,3-unsaturated and the 3,4-unsaturated compounds are the most active representatives of the series. Here the ability of a charged receptor group to polarize the unsaturated bond may be of importance. Since the longitudinal polarizability of these bonds is known to be larger than the transverse polarizability, the fact that the trans-2,3-doubly bonded compounds are more active than the cis-isomers may reflect the orientation to the receptor group that these agents can adopt. Thus the longitudinal component of the polarizability would be particularly large for the following conformation of the trans-isomer



which would correspond to the ACh conformation



No binding configuration for the cis-isomer, that conforms to the general stereochemical features of ACh appears to be suitably oriented for an optimum polarization of the double bond to occur.

There is conflicting evidence as to whether the ability of a compound to interact with the "carbonyl oxygen" site promotes muscarinic activity. The fact that the keto-alkyltrimethylammonium agents are less potent than the n-amyltrimethylammonium compound suggests that binding at the receptor group serves to decrease the activity. However, the low activity of D(–)-acetyl-β-methylcholine is presumably due to the disrupting effect the β-methyl group has upon the interaction of the carbonyl oxygen with the receptor group – an indication that binding at that site tends to increase the potency.

It has already been mentioned that methylfurtrethonium is considerably more potent a muscarinic agent than both the corresponding tetrahydrofuran compound and the ethyl choline ether. The larger activity was attributed to the ability of the furan ring to interact with the receptor cationic "carbonyl oxygen" site. Clearly the energy of binding involved would not lead to a greatly more stable agonist-receptor complex than in the case of the other two agents cited. It seems rather that the interaction serves to orient the furan ring so that it can effectively shield the cationic site.

The forces leading to a disruption of the agonist-receptor

complex were suggested to be ionic in nature. The receptor regions that would be most susceptible to their attack would therefore be either the anionic or the cationic site. Since the ability to bind at the former site is shared by all agonists, a perturbation of the binding would not lead to the observed differences in potencies. It seems rather that the receptor region that might be affected by the ionic forces is the cationic "carbonyl oxygen" group.

One may propose that it is the degree to which the binding of the agonist molecule at both the proton donor site as well as the cationic site serves to protect the cationic group from the impinging ionic forces that determines its muscarinic activity.

There is some evidence that the "carbonyl oxygen" binding group at nicotinic sites is a positively charged amino acid residue rather than a proton donor group. The fact that the nicotinic potencies of the 4-keto-amyltrimethylammonium compound and the 3-keto-amyltrimethylammonium compound are virtually the same indicates that the forces involved in binding the carbonyl groups of these agents do not depend strongly on the orientation of the interacting groups. The order of the activities of choline ethers

choline phenyl ether >> vinyl choline ether > ethyl choline ether

suggests that the ability of the receptor binding group to polarize these agents may be of importance. Similarly the fact

that the presence of an unsaturated bond in the alkyl-trimethylammonium compounds is associated with greater potency also seems to implicate a polarizing force. Finally the pronounced nicotinic activity of the compounds of the second class, compared to those of the first class, may also in part be due to the high polarizability of the carbonyl group.

It appears that the ability of compounds to interact with the "carbonyl oxygen" receptor group is directly related to their nicotinic activity, while the ability to bind at the ether oxygen site actually leads to a reduction in activity. Thus it has already been noted that the absence of an ether oxygen group, as in nicotine and its analogues or in the keto-alkyltrimethylammonium agents, is associated with a high nicotinic potency. On the other hand, the aliphatic choline ethers, which lack a group that can effectively interact with the "carbonyl oxygen" binding site but which do contain an ether oxygen, are even less active than the amyltrimethylammonium agent. In fact the least active representative of that series is ethyl choline ether, which would be bound most strongly at the "ether oxygen" site.

These trends would lead one to expect that in the compounds that can bind at both sites the relative strengths of the interactions would determine the nicotinic activity. Thus a sensitive balance of the attractive forces to either binding site may be involved. In the compounds of the first class the

hydrogen bonding interaction would dominate, while in the compounds of the second class the attraction to the positively charged receptor group would be stronger.

Invoking a similar reasoning to that used above, it may be stated that two factors determine the nicotinic activity of an agent: the degree to which its binding at the "carbonyl oxygen" site serves to shield that receptor group from the disruptive ionic forces, as well as the degree to which its binding at the "ether oxygen" site does not promote the action of those forces.

The aromatic choline ethers may owe their pronounced activity to the fact that, while the ether oxygen of these agents can be strongly bound, the size of the phenyl group might be sufficiently large to nevertheless protect the cationic site.

4) Accessory Binding Sites and Steric Fit

The potency promoting effect of the terminal methyl group of agents in classes one and two has been attributed to the binding of that group, its steric fit, and its role in the desolvation step. It seems that none of these explanations are entirely adequate to explain the large reduction in muscarinic activity that is associated with the replacement of the group by a hydrogen.

The presence of the terminal methyl function may serve to protect the receptor active site from the approach of ions. The low muscarinic potencies of the bulkier choline esters, as well as the two dimethyl analogues of muscarine, methyldilvasene and muscarone, suggest the muscarinic site is very compact. The

terminal methyl group of potent agonists may occupy a strategic position at the receptor binding cavity.

It has already been noted that epimuscarine is considerably less active than muscarine, and that desmethyl epimuscarine is almost equiactive with epimuscarine. Perhaps the binding arrangement that leads to an optimum binding of the two oxygen functions of epimuscarine also involves a displacement of the 2-methyl group from the strategic position accessible to the 2-methyl group of muscarine. As a result the receptor binding compartment might become just as susceptible to the attack of ions as if the methyl group were absent.

In the preceding discussion it was proposed that the nicotinic binding site considerably resembles the muscarinic site in its characteristic features. However the relatively high nicotinic activities of bulky choline esters and ethers suggest that the corresponding compartment is not very compact. The pronounced potencies of the aromatic choline esters may even reflect the presence of accessory binding sites for the conjugated rings of these molecules.

Structure of the Active Site

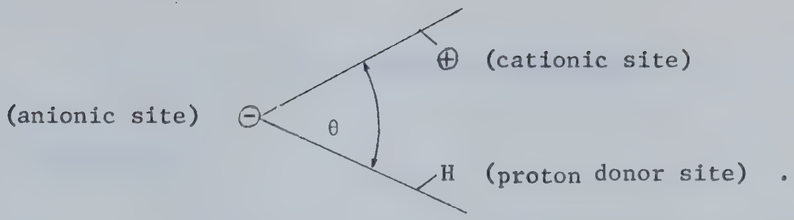
The same type of binding groups have been suggested to be present at both muscarinic and nicotinic sites: an anionic group, a proton donor group and a cationic group. There is no reason to assume, however, that the amino acid residues that

constitute these groups are identical chemical species at both types of receptor active site.

It was proposed that, with the exception of the choline phenyl ethers, the ability of agents to bind at the proton donor site is associated with a decrease in nicotinic activity. The difference in the nicotinic action of the compounds of the first and second classes was attributed to a difference in their relative strengths of binding at the proton donor site and the cationic site. The pronounced muscarinic activity of these agents was suggested to be related to their ability to bind at both sites.

An explanation of the different effects of the hydrogen bonding interaction may be offered if one assumes that the nicotinic and the muscarinic sites do not have the same structure.

Since the portion of agonist molecules most strongly bound is presumably the cationic head group, the receptor anionic region may constitute a primary anchoring site. The only degree of freedom left for the interaction of an agonist molecule with the remaining binding groups would consist in a rotation about that point of attachment. The main difference in the construction of the nicotinic and muscarinic binding compartments might therefore lie in the angle θ that subtends between the proton donor group and the cationic group


(cationic site)
(anionic site)
θ
H
(proton donor site)

At muscarinic receptors the angle would be such that near optimum binding of agonists at both these groups can occur. At nicotinic receptors it would be larger, so that a competition for binding at the two sites would take place.

V. THEORETICAL TREATMENT

Introduction

In the previous Chapter an attempt was made to determine the nature of the region responsible for the binding of agonists at cholinergic receptors. It was suggested that the following binding groups are present at both muscarinic and nicotinic sites: an anionic group, a proton donor group, and a cationic group. Further it was proposed that the activity of agonists is related to the degree to which the interaction of these agents with the latter two receptor groups serves to stabilize the agonist-receptor complex. As was indicated, the stabilization would in part be determined by the strength of the binding, and also indirectly by the amount to which this binding leads to a localization of certain portions of the agonist molecule at strategic positions at the receptor active site.

In this Chapter the theoretical method described in Chapter II will be used to investigate the interaction of select agonist molecules with the postulated receptor binding groups. It is within the scope of this approach to determine the energy diagrams corresponding to the linear approach of two molecules (interaction energy vs. separation), as well as to the rotation of one molecule with respect to another (interaction energy vs. angle). In the following treatment both types of diagrams will be utilized.

The "linear approach" curves for the interaction of a number of select agonists with hypothetical receptor components will be studied and the energies and separations corresponding to the minima will be determined. The results will be used to investigate the degree to which an agent's ability to bind at the receptor groups is related to its potency.

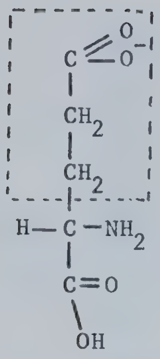
Curves corresponding to the rotation of an agonist about its quaternary nitrogen, with respect to a fixed receptor group, will be presented, in order to estimate the torque resulting from the forces acting on the agonist molecule.

It should be noted that the perturbing effects of the ionic environment, as well as of the receptor portions that are not part of the active site, are neglected in these calculations.

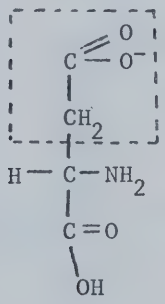
Preliminary Considerations

So far the components of the receptor active site have been described rather schematically. For the purpose of carrying out quantum mechanical investigations it is necessary to consider in detail what amino acid residues may constitute these binding groups. Certain proposals pertaining to this question were already made in the previous Chapter.

It was suggested that in the case of the receptor anionic function the carboxylate ion of the conjugate base of L-glutamic acid

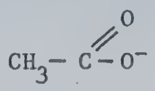


may be involved. However it is also possible that the carboxylate ion of the conjugate base of L-aspartic acid



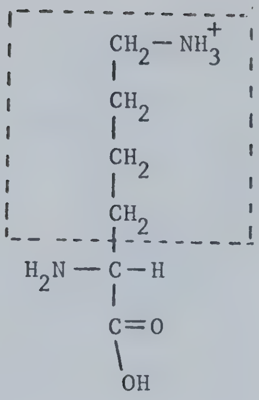
constitutes that group.

In both cases the same amino acid segment would be responsible for the strong interaction with the agonist cationic groups. It will be assumed that the acetate ion

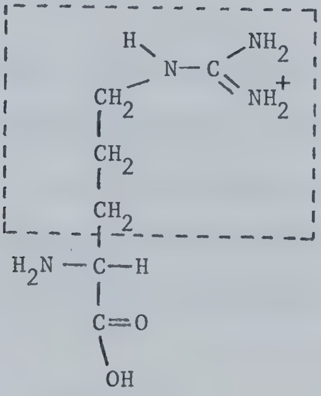


is a suitable model system for representing the significant portions of the two amino acids.

The positively charged receptor binding group is probably either the side chain of the amino acid L-lysine



or the side chain of L-arginine

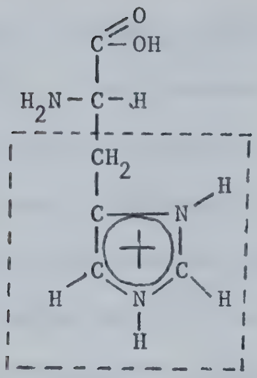


The methylammonium ion

$$CH_3-NH_3^+$$

will be chosen to represent the two positively charged side chains.

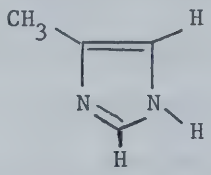
There are a number of amino acid residues that might constitute the receptor proton donor group. It has already been mentioned that the conjugate acid of L-histidine



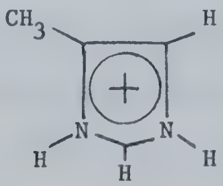
frequently performs the role of a proton donor at enzyme active sites.

In view of the limited scope of this work a calculation of the interaction of various agonists with all the eligible hydrogen bonding groups - some of which can occur in several stages of activation depending on the neighboring molecules - seems rather unwarranted. Instead a different avenue of approach will be adopted.

The compound 4-methylimidazole,



which resembles the side chain of histidine, will be used as a prototype for weak proton donors, while its conjugate acid



will serve as a prototype for strong proton donors.

An examination of Fig. I, which depicts the charge distributions of both forms, will indicate that the choice is a reasonable one. The 1-nitrogen of 4-methyl imidazole is negatively charged, which suggests a weaker hydrogen bonding capacity for that center than in the case of the conjugate acid, where the corresponding nitrogen carries an effective positive charge.

Another aspect, that needs to be clarified, concerns the conformation of the agonist molecules at the receptor active site.

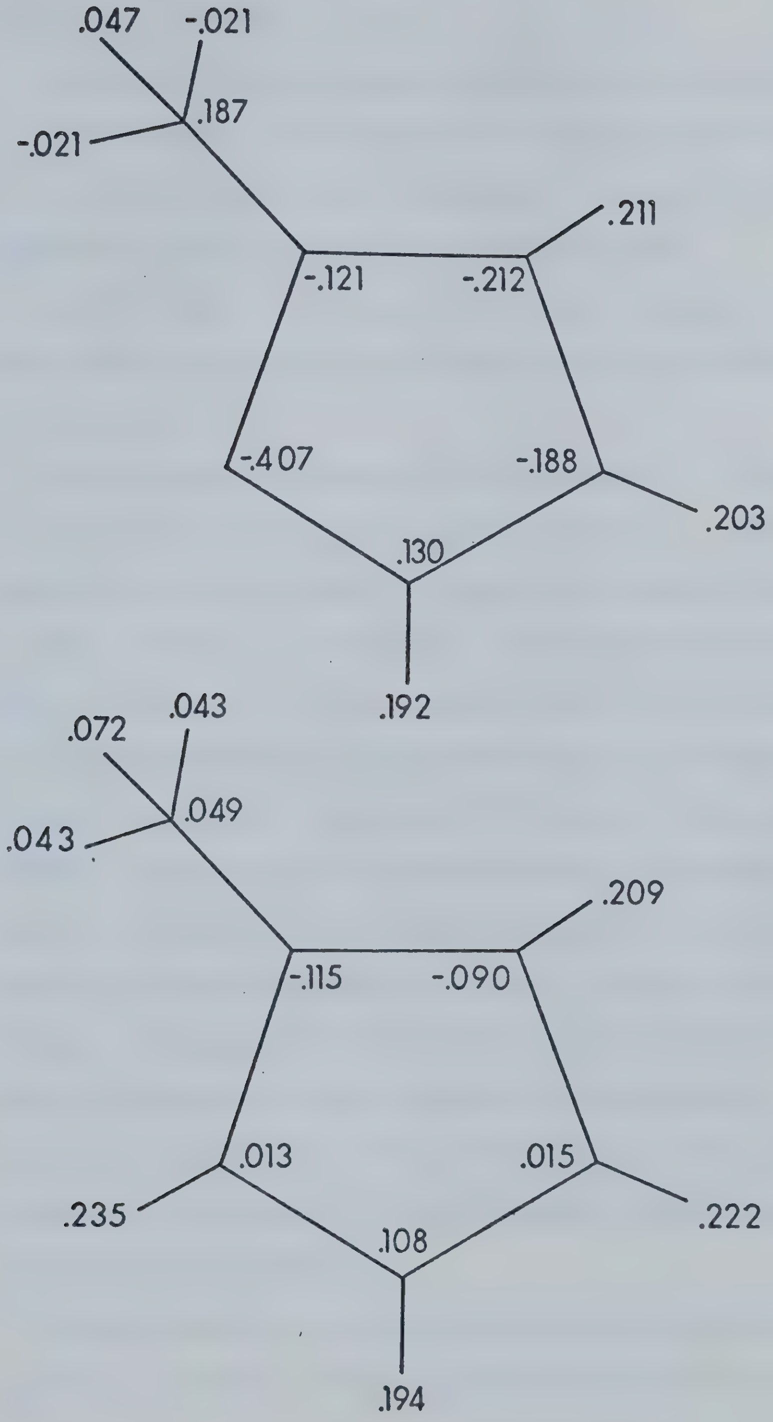
X-ray crystallography has been employed by several workers [126,127] to elucidate the structure of cholinomimetic agents in the crystalline state. Of the molecules to be investigated in this work only acetylcholine [128], L(+)-muscarine, and cis-2-(S)-methyl-4-(R)dimethylaminomethyl-1,3-dioxolane methiodide [129] have been studied using that technique.

The relevance of this structural data for considering the conformation of the receptor-bound compounds may be seriously questioned. The conformation of an agonist molecule at the receptor may be thermodynamically less stable than that adopted in the solid state.

Quantum mechanical calculations of the preferred conformations of a number of agonists have also been carried out.

Beveridge and Radna [130] have investigated the potential energy surface for the rotation about two of the bonds of ACh using the INDO method. They were able to suggest several stable

FIG. I


.047
-.021
.187
-.021
.211
-.121
-.212
-.407
-.188
.203
.130
.192
.072
.043
.049
.043
.209
-.115
-.090
.013
.015
.235
.222
.108
.194

conformations for that molecule.

Similar calculations using the EHT method have led Kier [131, 132] to propose preferred conformations for L-muscarine and D-muscarone. In both cases the side chains containing the quaternary ammonium function are predicted to be nearly fully extended.

Again it may be questioned whether these calculated structures, which refer to the molecules in vacuum, reflect the conformation at the receptor site.

The following conformations were chosen for the compounds for which calculations were performed in this study. The geometry of the ring systems of muscarine and methyldilvasene was based upon the crystallographic structures, while a fully extended conformation of the substituent chains was adopted. The geometries of ACh and methylfurtrethonium were adjusted to fit that of muscarine. The optimum coincidence of the quaternary nitrogen, "ether" oxygen and terminal methyl group positions was used as a criterion. Fig. II shows the degree of coincidence that exists between the structures of ACh, muscarine, and methyldilvasene. The geometries of the other agents for which calculations were carried out, were based upon the ACh geometry. Standard bond lengths and bond angles [133] were used to determine the structure of molecule portions for which crystallographic results were not available.

Molecular models were used to select paths of approach for the interacting molecules, that do not involve excessive steric

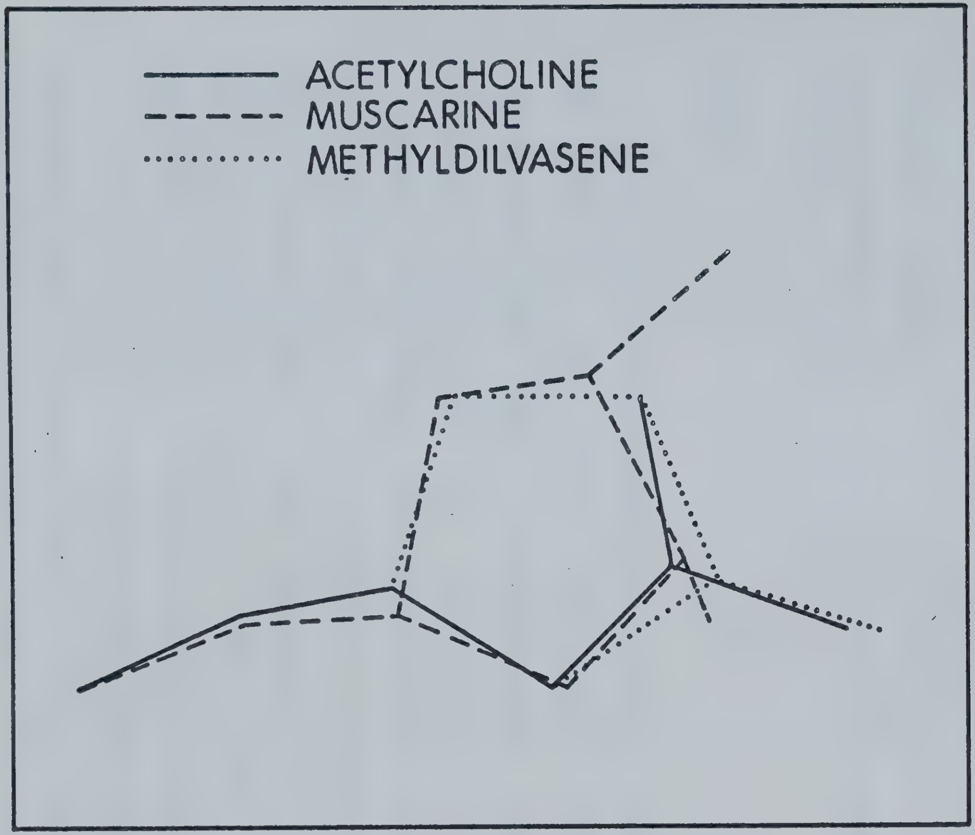

ACETYLCHOLINE
MUSCARINE
METHYLDILVASENE

FIG. II

STRUCTURAL SIMILARITIES

hindrance. Since the orientation of the receptor groups to the bound agonist molecule need not correspond to the optimum orientation for binding, no attempt was made to determine the configuration corresponding to the minimum energy. Instead, in certain cases, the calculations for two different binding configurations were performed, in order to estimate the degree to which a change in the orientation of the interacting molecules influences the relative order of the binding energies.

Results

Calculations using the AVE SCF method of Fraga and Carbo were performed for ACh, muscarine, methyldilvasene, methylfurtrethonium, ethyl choline ether, demethyl ACh, didemethyl ACh, tridemethyl ACh, the acetate ion, the methylammonium ion, the quarternary methylammonium ion, 4-methyl imidazole, and the conjugate acid of 4-methyl imidazole. The bond order matrices, the orbital energies for the highest occupied orbital, and the average excitation energies for occupied to virtual orbital excitations were utilized as input data for the molecular interaction program.

The interaction energy diagram corresponding to the linear approach of ACh to an acetate ion (Fig. III) was determined. The resulting curves for the component energies and the total interaction energy are depicted in Fig. IV. The electrostatic interaction energy constitutes the largest contribution at positions close to the minimum, and the position of the minimum for the

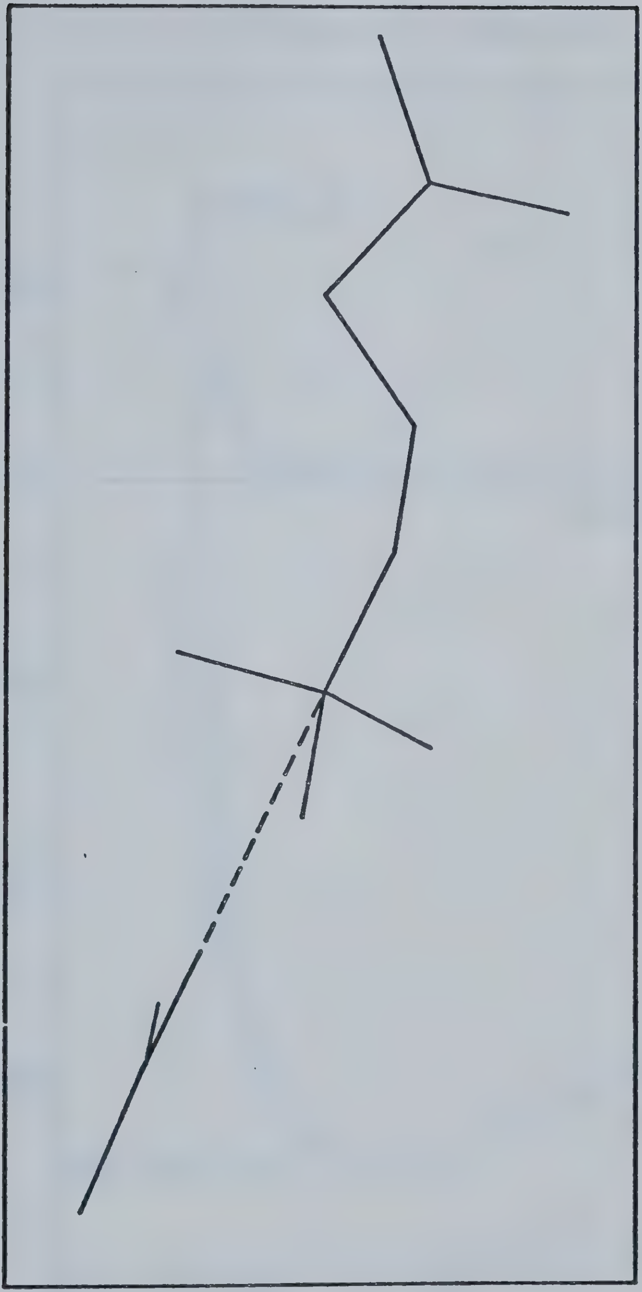
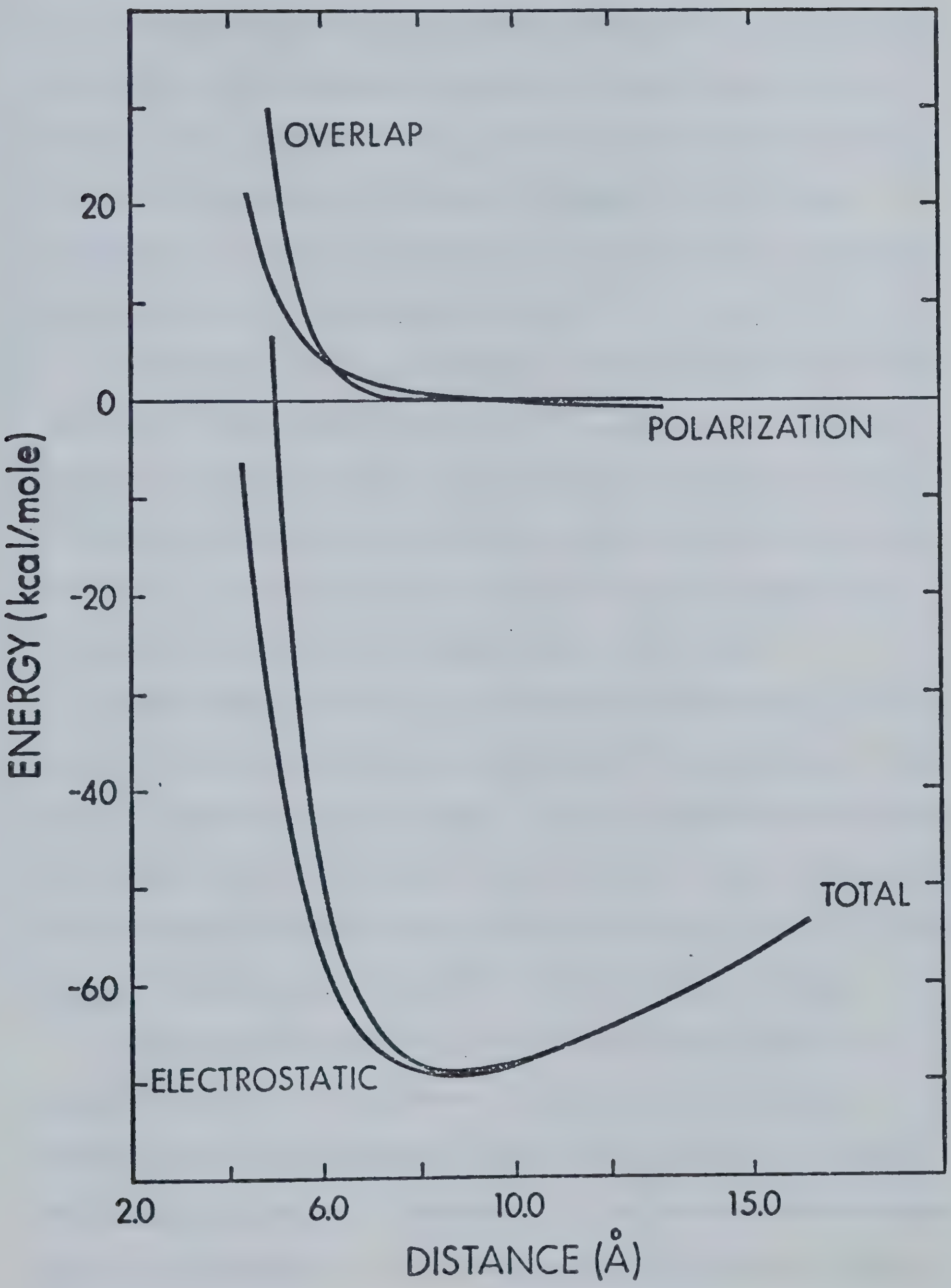


FIG. III

ACH-ACETATE ION INTERACTION

FIG. IV

ACH-ACETATE ION INTERACTION



electrostatic energy curve nearly coincides with that for the total energy curve.

Consequently it was considered sufficient to calculate only the electrostatic component energies for the interactions of demethyl ACh, didemethyl ACh, and tridemethyl ACH with an acetate ion. The same mode of approach was chosen as in the above calculation. The resulting curves are shown in Fig. V, and the pertinent data is summarized in Table I.

The interaction energy diagrams corresponding to the linear approach of the compounds ACh (Fig. VI), muscarine, methyldilvasene, methylfurtrethonium and ethylcholine ether to 4-methyl imidazole, at two orientations, and to its conjugated acid were determined. The order of the calculated binding energies was found to be essentially the same in all three cases.

Calculations for the interactions with the conjugate acid of 4-methyl imidazole were not carried out for separations of less than 3 Å between the key centers, since the polarization contribution was found to be very large in that range. This rather suggests that the error due the neglect of the polarization in the calculation of the overlap energies may also be relatively large.

The two orientations chosen for the imidazole ring are shown in Fig. VI. Orientation (2) represents the optimum orientation for the hydrogen bonding of the proton donor group with the ether oxygen of muscarine. It is proposed that orientation (1) corresponds to the configuration for which the change in

FIG. V

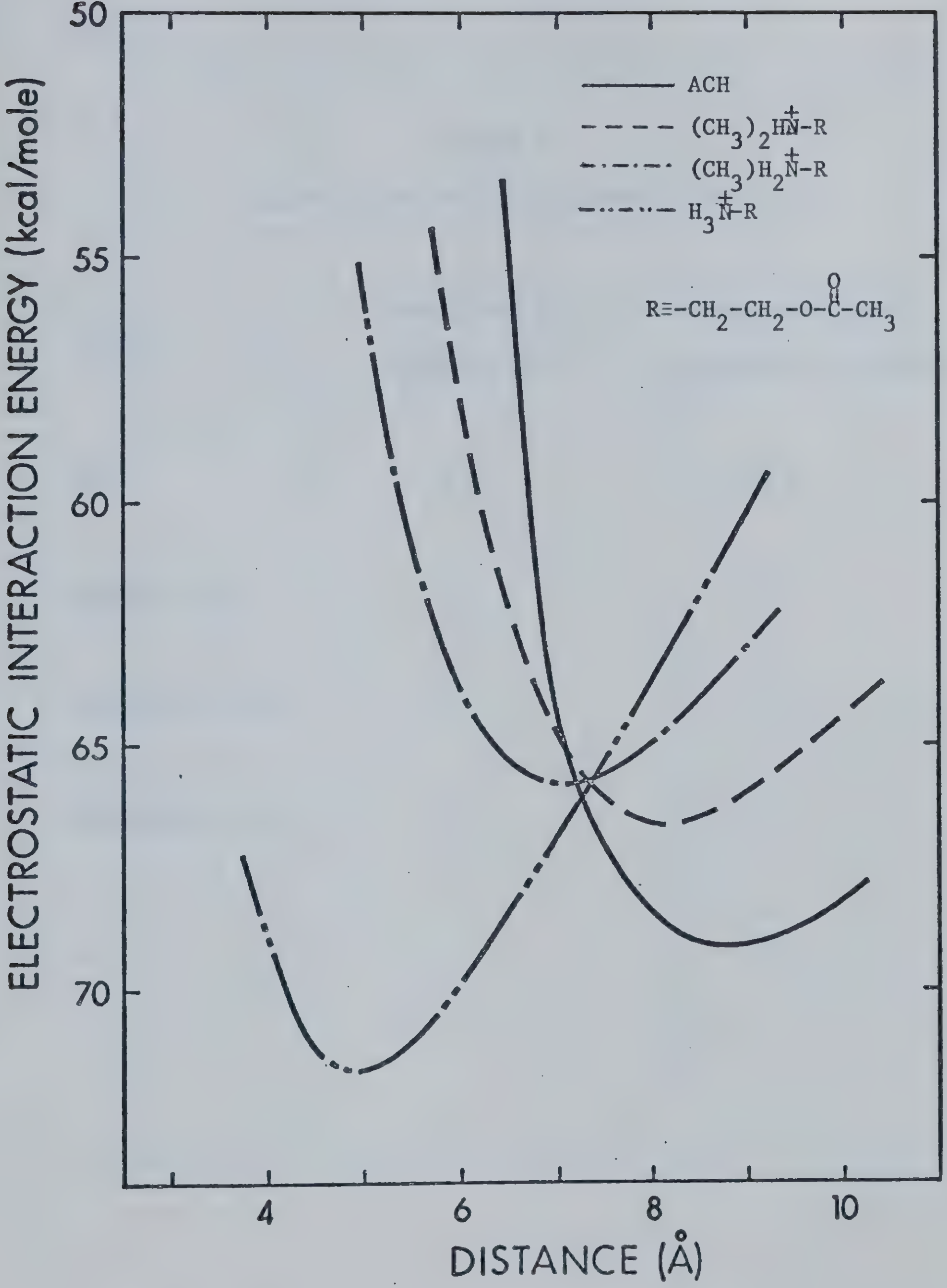

ACH
(CH3)2HN+-R
(CH3)H2N+-R
H3N+-R
R≡-CH2-CH2-O-C(=O)-CH3
50
55
60
65
70
4
6
8
10
ELECTROSTATIC INTERACTION ENERGY (kcal/mole)
DISTANCE (Å)

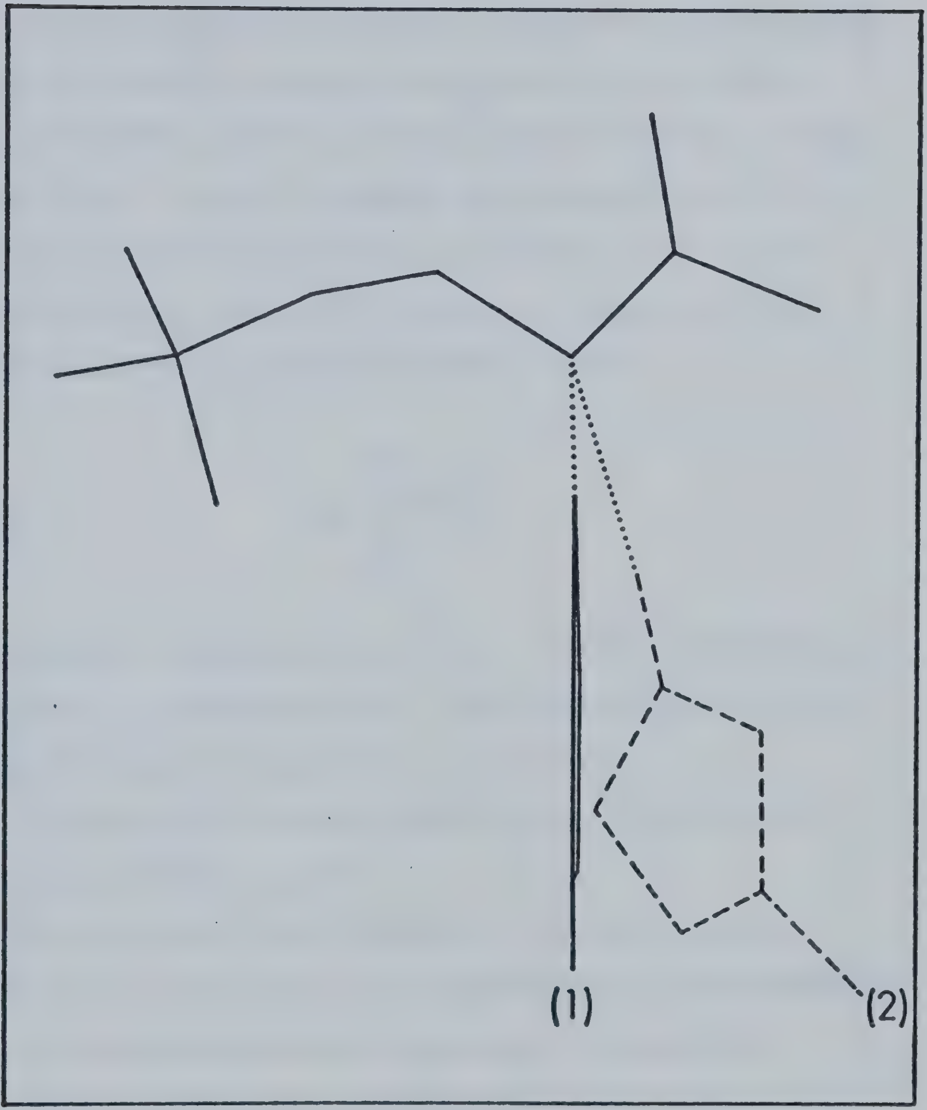
TABLE I.

INTERACTIONS WITH THE ACETATE ION.

SYSTEM	SEPARATION AT MINIMUM (Å)	ELECTROSTATIC ENERGY AT MINIMUM (KCAL/MOLE)
ACH	8.7	-69.6
DEMETHYL ACH	8.1	-66.6
DIDEMETHYL ACH	7.1	-65.8
TRIDEMETHYL ACH	4.5	-71.1

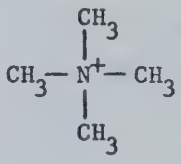
FIG. VI

ACH-4-METHYL IMIDAZOLE INTERACTIONS



the force of attraction between the agonist and the proton donor group, for a rotation about the quaternary nitrogen center, is least rapid.

The curves showing the component energies for the interaction of ACh with 4-methyl imidazole (orientation (2)) are given in Fig. VII, and the curve for the total interaction energy is shown in Fig. VIII. In order to estimate the contribution due to the interaction between the quaternary ion portion of the agonist molecule and the proton donor species, a calculation of the interaction of the quaternary methylammonium ion



$$N^{+}(CH_3)_4$$

with 4-methyl imidazole was carried out. The same mode of approach was used as in the previous case. The resulting curves for the component energies are depicted in Fig. IX.

The results for the proton donor-agonist interactions are summarized in Tables II - IV.

The interaction energy diagrams for the linear approach of ACh (Fig. X), muscarine, and methyldilvasene to a methylammonium ion, at two orientations, were determined. Orientation (1) corresponds to the optimum approach of the latter ion to one of

FIG. VII

ACH-4-METHYL IMIDAZOLE INTERACTION

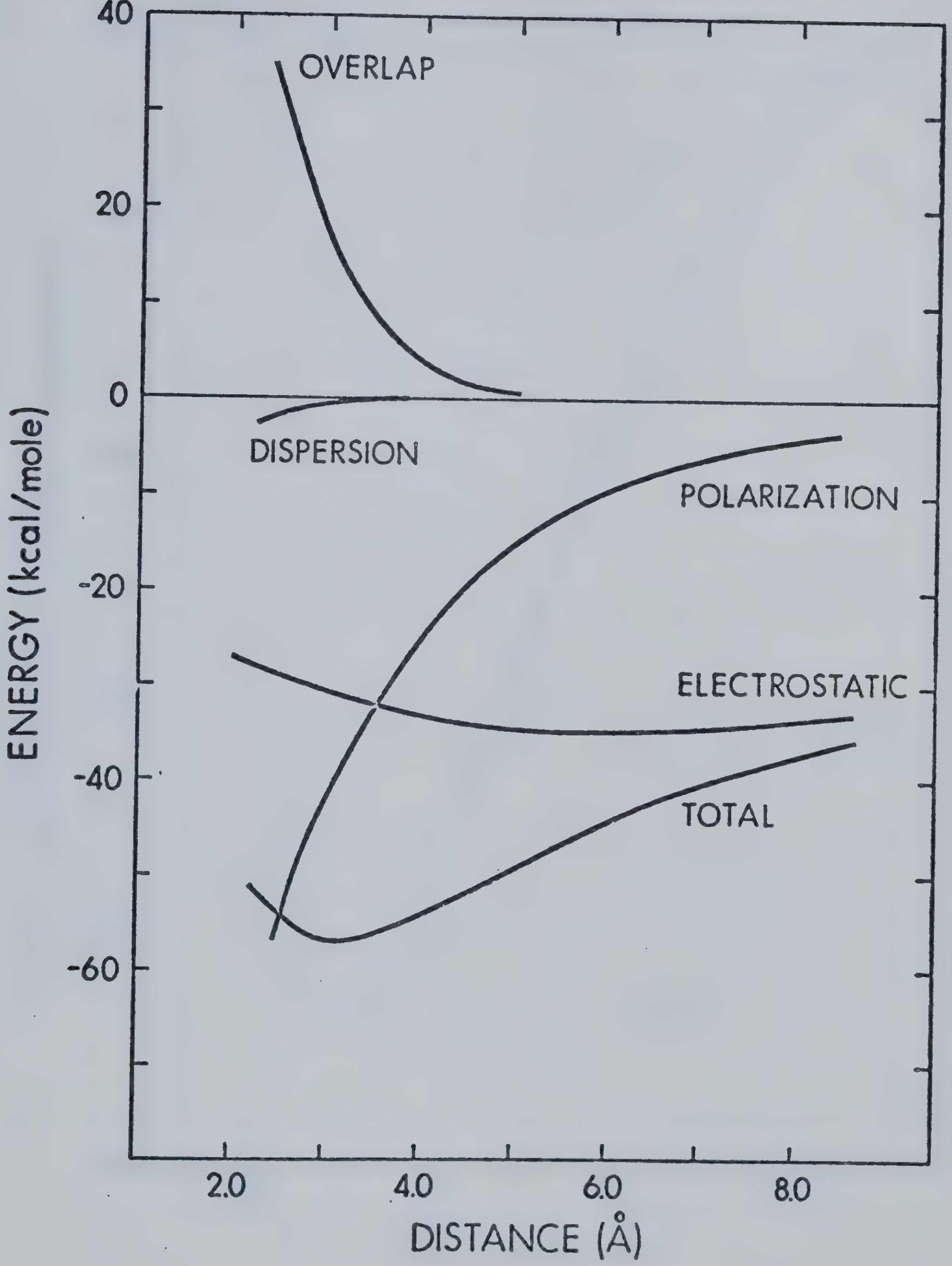


FIG. VIII

ACH-4-METHYL IMIDAZOLE INTERACTION

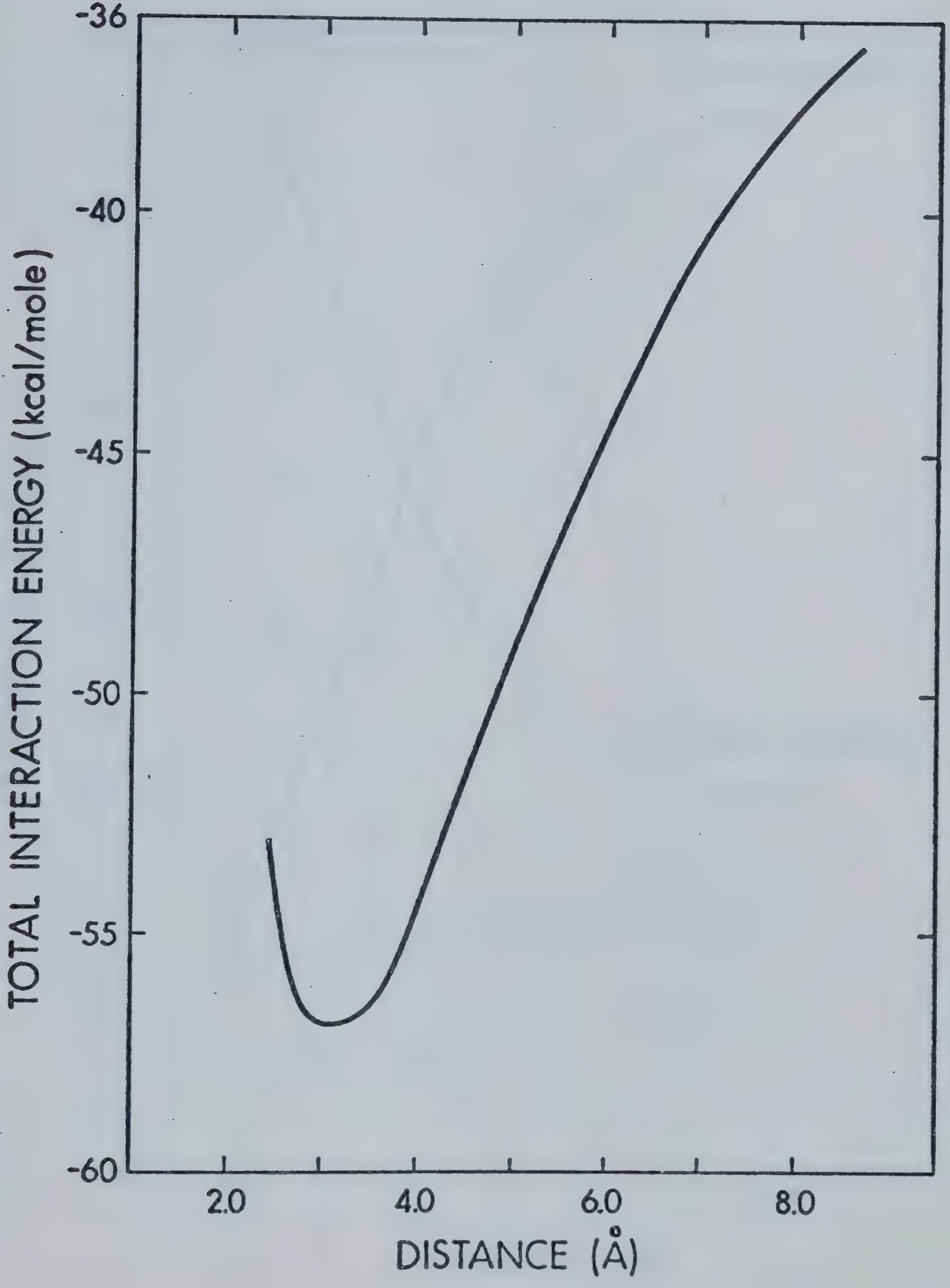


FIG. IX

4-METHYL IMIDAZOLE-QUATERNARY METHYLAMMONIUM INTERACTION

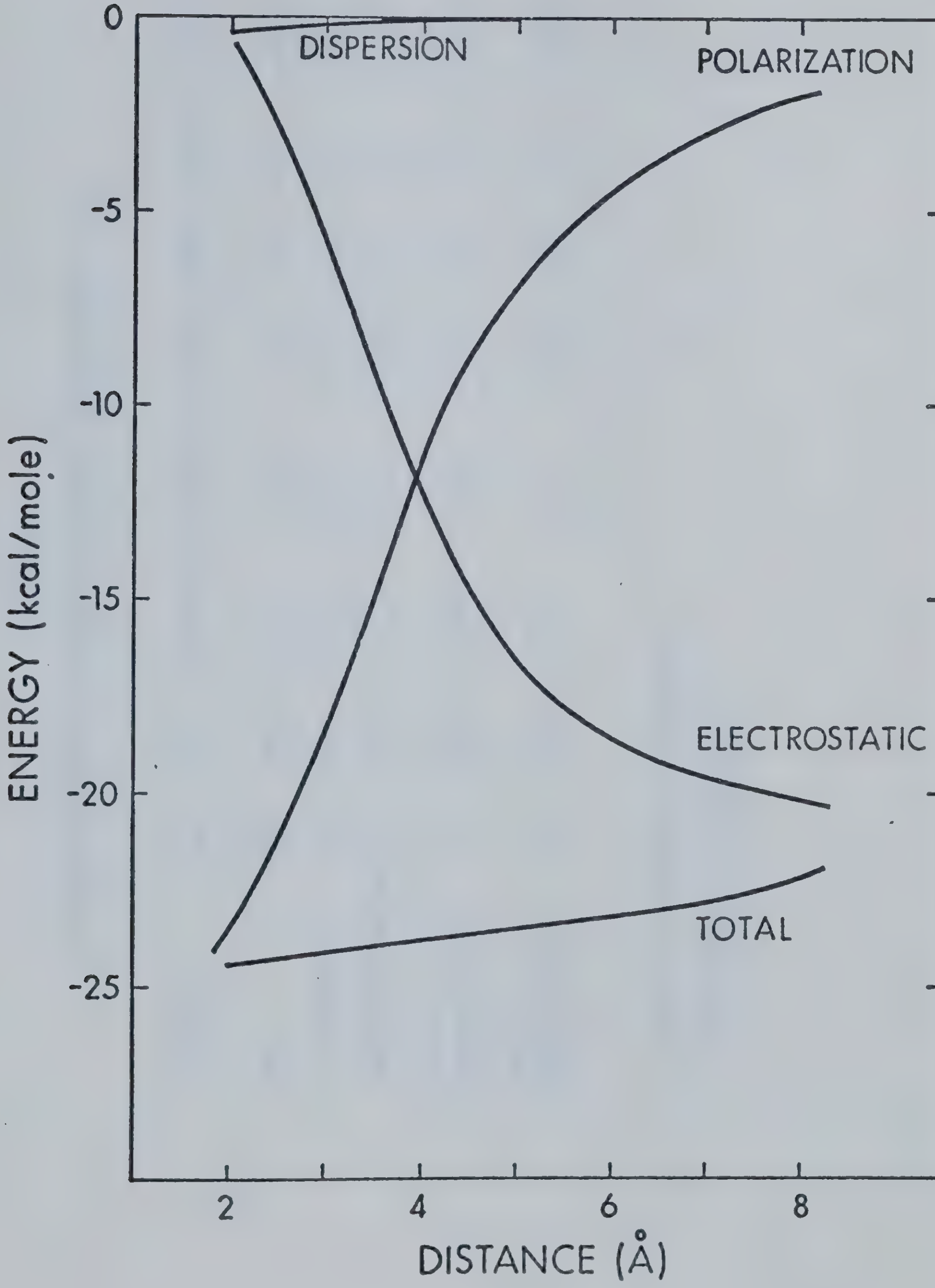


TABLE II.

INTERACTIONS WITH 4-METHYL IMIDAZOLE (ORIENTATION (1))

SYSTEM B	R_{min} †	EEL*	EPOL(A)*	EPOL(B)*	EPOL*	EDISP*	EOVLP*	ETOT*
ACH	3.7	-28.0	-24.7	-5.8	-30.5	-0.3	9.2	-49.7
L-MUSCARINE	4.0	-33.6	-23.3	-4.5	-27.8	-0.2	6.3	-55.3
METHYLDILVASENE	4.0	-32.5	-22.2	-4.3	-26.5	-0.2	6.6	-52.7
METHYL-FURTRETHONIUM	4.2	-29.0	-23.3	-3.9	-24.2	-0.3	7.1	-46.6
CHOLINE-ETHYL ETHER	4.8	-29.6	-14.3	-2.3	-16.6	-0.1	3.4	-43.5

* ENERGY AT MINIMUM IN KCAL/MOLE

† SEPARATION AT MINIMUM IN Å

TABLE III.

INTERACTIONS WITH 4-METHYL IMIDAZOLE (ORIENTATION (2))

SYSTEM B	R_{min} †	EEL*	EPOL(A)*	EPOL(B)*	EPOL*	EDISP*	EOVLP*	ETOT*
ACH	3.0	-30.5	-30.4	-12.0	-42.4	-0.8	16.7	-56.9
L-MUSCARINE	3.0	-33.4	-30.7	-11.9	-42.6	-0.7	11.9	-64.8
METHYLDILVASENE	3.6	-33.3	-25.6	-6.5	-32.2	-0.5	9.5	-56.5
METHYL-FURTRETHONIUM	4.0	-26.8	-24.8	-5.3	-30.1	-0.3	6.0	-51.2
ETHYL-CHOLINE ETHER	4.25	-29.8	-16.5	-3.3	-19.8	-0.2	3.6	-46.2

* ENERGY AT MINIMUM IN KCAL/MOLE

† SEPARATION AT MINIMUM IN Å

TABLE IV.

INTERACTIONS WITH THE CONJUGATE ACID OF 4-METHYL IMIDAZOLE (ORIENTATION (1))

SYSTEM B	R_{min} †	EEL*	EPOL(A)*	EPOL(B)*	EPOL*	EDISP*	EOVLP*	ETOT*
ACH	3.0**	29.7	-27.3	-48.9	-76.2	-1.0	21.6	-25.9
L-MUSCARINE	3.0**	26.6	-28.2	-48.7	-76.9	-0.9	21.3	-29.8
METHYLDILVASENE	3.0**	25.4	-26.6	-46.5	-73.2	-1.1	22.1	-26.7
METHYL-FURTRETHONIUM	3.0**	46.3	-36.1	-52.1	-88.2	-1.3	25.2	-18.0
ETHYL-CHOLINE ETHER	4.2	19.1	-16.2	-20.1	-36.3	-0.3	5.7	-11.6

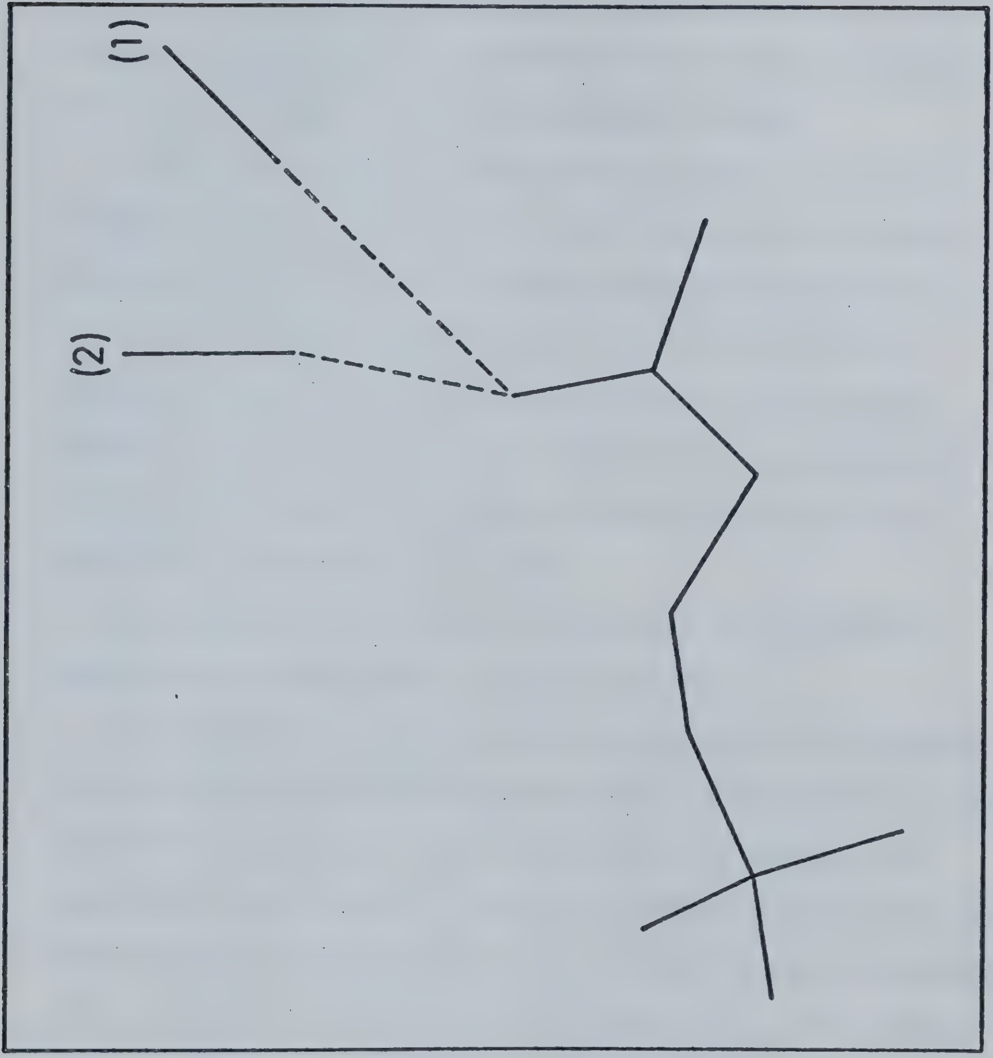
* ENERGY AT MINIMUM IN KCAL/MOLE

† SEPARATION AT MINIMUM IN Å

** CALCULATIONS NOT CARRIED OUT FOR R < 3.0

FIG. X

ACH-METHYLAMMONIUM ION INTERACTION



the ACh carbonyl oxygen lone pairs. Orientation (2) represents a path that is nearly co-linear with the carbonyl carbon-oxygen bond, and could thus be expected to lead to the largest polarization of that bond. For the same reason that was given above, the calculations were not performed for separations of less than 3 Å between the carbonyl oxygen and the methylammonium nitrogen.

Since all of the interacting molecules have a net positive charge most of the curves for the total interaction energy show increasing repulsion with decreasing intermolecular separation. In order to estimate the contribution due to the interaction between the quaternary portion of the agonists and the methylammonium ion, calculations of the interaction of the quaternary methylammonium ion with the latter ion were carried out for the same modes of approach as used above.

The results for the various interactions with the methylammonium ion are summarized in Tables V and VI.

The interaction energy diagrams corresponding to the rotation of ACh, muscarine and methyldilvasene about their quaternary nitrogen centers were determined. The curves reflect the change in the interaction energy resulting from the rotation away from either the methylammonium ion (orientation (2)), 4-methyl imidazole (orientation (1)), or from its conjugate acid (orientation (1)). The unrotated configuration of the interacting molecules was chosen to be the same as that for the linear approach - at the separation corresponding to the minimum in the case of 4-methyl imidazole, and

TABLE V.

INTERACTIONS WITH THE METHYLAMMONIUM ION (ORIENTATION (1))

SYSTEM B	R_{min}[†]	EEL*	EPOL(A)*	EPOL(B)*	EPOL*	EDISP*	EOVLP*	ETOT*	
								(a)††	(b)
ACH	3.0**	31.9	-4.9	-25.4	-30.5	-0.3	12.5	13.6	-17.3
L-MUSCARINE	4.8	12.7	-2.1	-5.9	-8.0	0.0	0.8	5.5	-15.3
METHYLDILVASENE	3.0**	32.5	-4.1	-25.2	-29.3	-0.5	11.2	14.0	-16.9

* ENERGY AT MINIMUM IN KCAL/MOLE

† SEPARATION AT MINIMUM IN Å

** CALCULATIONS NOT CARRIED OUT FOR R < 3.0

†† (a) TOTAL INTERACTION ENERGY

(b) CORRECTED TOTAL INTERACTION ENERGY

TABLE VI.

INTERACTIONS WITH THE METHYLAMMONIUM ION (ORIENTATION (2))

SYSTEM (B)	R_{min} †	EEL*	EPOL(A)*	EPOL(B)*	EPOL*	EDISP*	EOVLP*	ETOT*	
								(a)††	(b)
ACH	3.0**	26.0	-2.2	-26.1	-28.3	-0.2	2.1	-0.4	-35.3
L-MUSCARINE	3.0**	32.5	-2.5	-18.6	-21.1	-0.4	8.8	19.9	-16.3
METHYLDILVASENE	3.0**	33.6	-3.0	-23.8	-26.8	-0.5	7.2	13.6	-20.0

* ENERGY AT MINIMUM IN KCAL/MOLE

† SEPARATION AT MINIMUM IN Å

** CALCULATIONS NOT CARRIED OUT FOR R < 3.0

†† (a) TOTAL INTERACTION ENERGY

(b) CORRECTED TOTAL INTERACTION ENERGY

FIG. XI

ACH-4-METHYL IMIDAZOLE(CONJUGATE ACID) INTERACTION

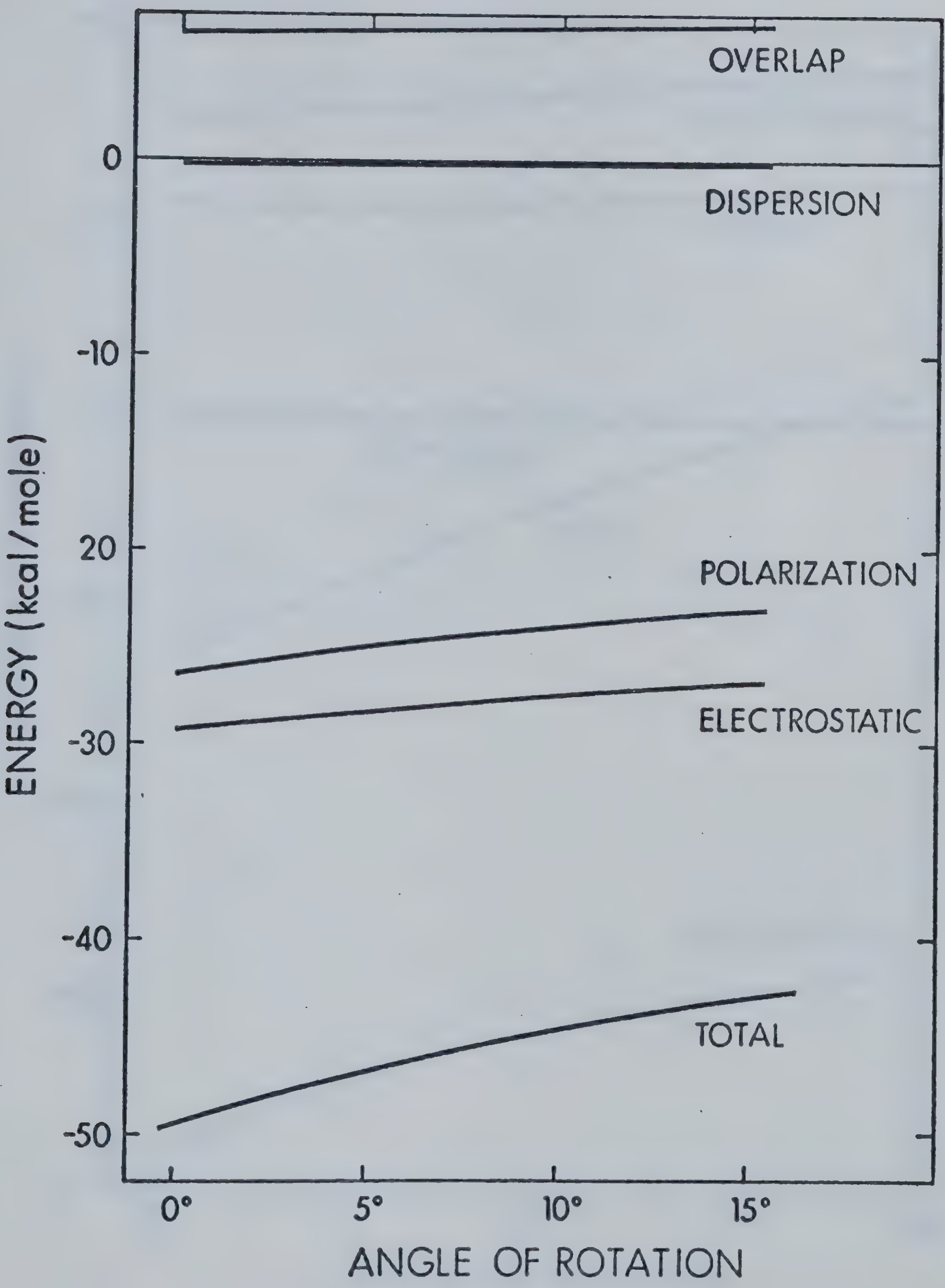


FIG. XII

ACH-4-METHYL IMIDAZOLE INTERACTION

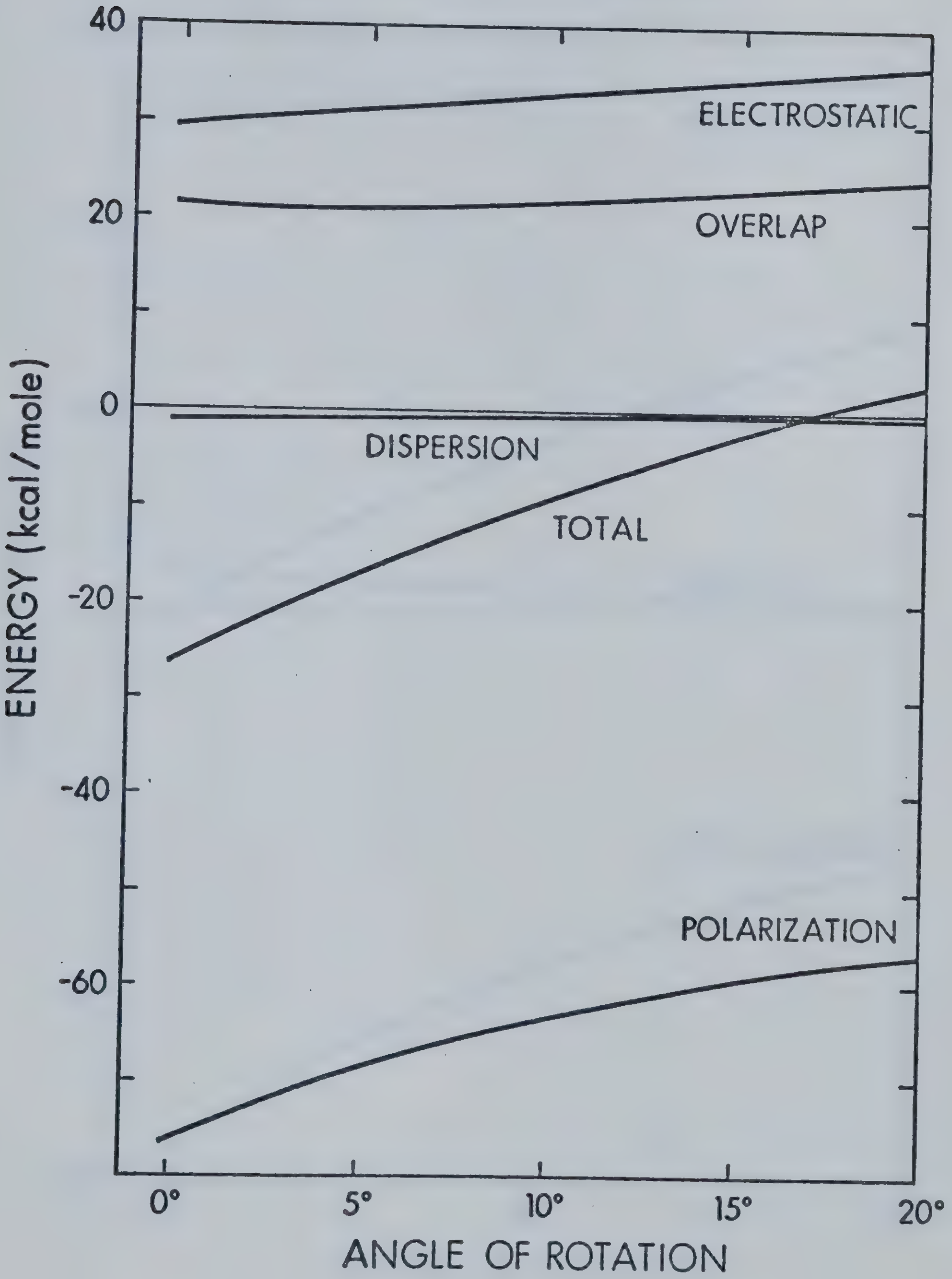


FIG. XIII

ACH-METHYLAMMONIUM ION INTERACTION

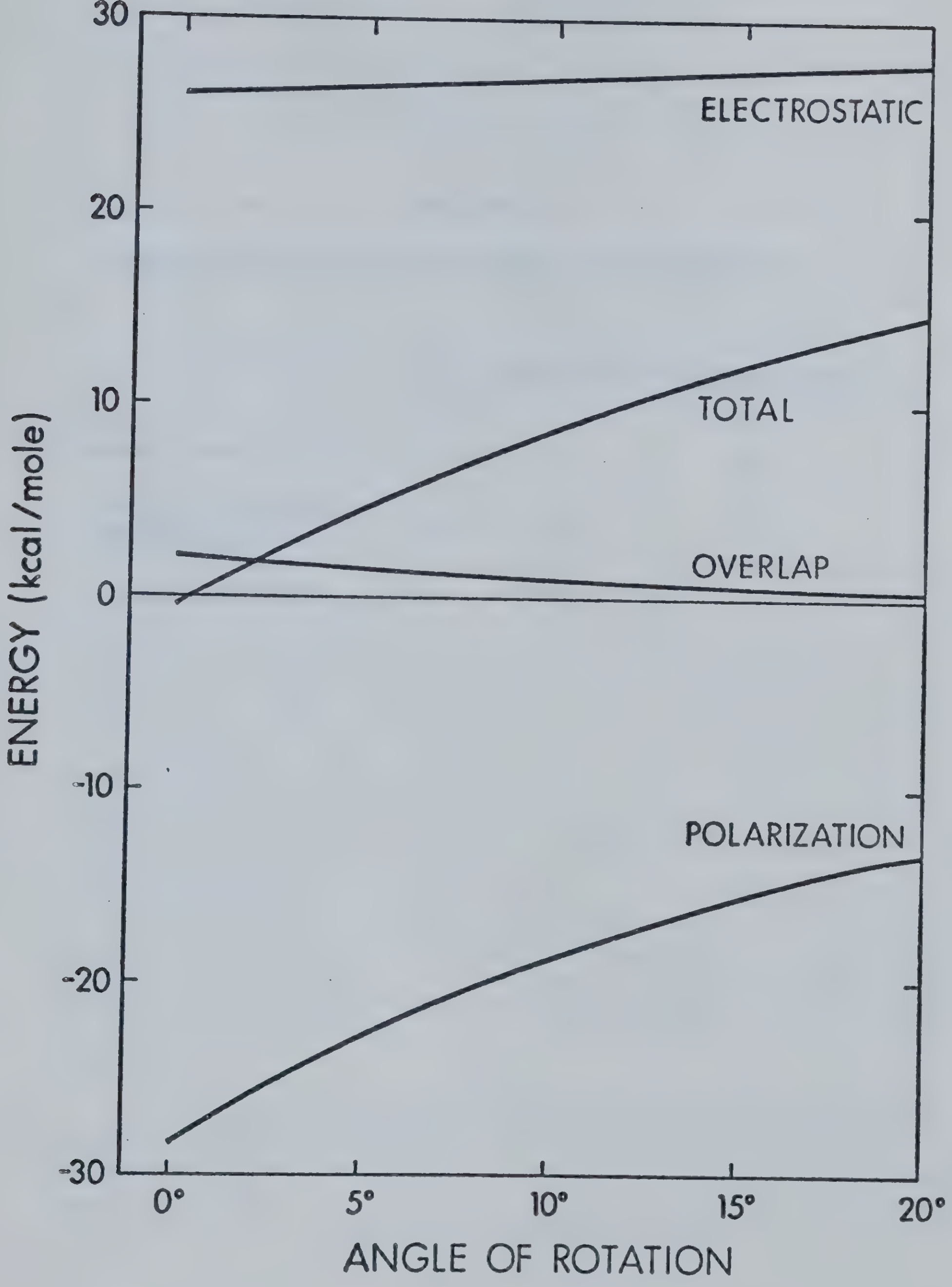


TABLE VII.

MAXIMUM SLOPES OF ROTATION CURVES (IN KCAL/DEGREE).

	ACH	L-MUSCARINE	METHYLDILVASENE
METHYLAMMONIUM ION	1.0	-0.1	0.7
4-METHYL IMIDAZOLE CONJUGATE ACID	1.8	1.4	1.4
4-METHYLIMIDAZOLE	0.5	0.4	0.5

at a separation of 3 Å in the other two cases.

The resulting curves for the component energies, and the total energy for the interactions with ACh are depicted in Figs. XI - XIII. The pertinent data is summarized in Table VII.

Discussion

Before attempting to relate the calculated results to the pharmacological properties of the various agonists involved, it may be desirable to examine in detail the specific features of each of the interaction processes.

(1) Interactions with the Acetate Ion

In the model for the AChR binding compartment put forth in Chapter IV, the anionic group was portrayed as a primary anchoring site for the agonist molecules. The results for the ACh-acetate ion interaction (Fig. IV) show that, while the binding energy is indeed very large, the curve for the total interaction energy is quite shallow near the minimum. This would indicate that the force constraining the ACh molecule at the separation corresponding to the minimum is not very great, and that small displacements to facilitate the binding of the agent at the other sites may readily take place.

It was noted that the stepwise replacement of the methyl groups of the quaternary ammonium function of agonists by hydrogens leads to a steady and rapid decrease in activity. One explanation for this trend was that demethylation leads to a closer binding

distance for the interaction with the receptor anionic group. This was suggested to entail a less optimal binding for the interaction with the other receptor groups.

Fig. V shows that there is indeed such a shift in the position of the minima for the interaction of the various demethylated analogues of ACh with an acetate ion. The closer binding is presumably attributable to the increasing localization of positive charge on the quaternary nitrogen, which leads to a stronger electrostatic attraction to the anion, as well as to a reduction in the overall steric repulsion due to the methyl groups. In addition demethylation also entails a decrease in binding energy (see Table I) for the first two demethylated forms, while the tridemethylated ACh analogue is actually bound more strongly than ACh. Further it is noteworthy that the curves (Fig. V) become progressively less shallow near the minimum with the removal of methyl groups. This suggests that the force constraining an agent at the separation corresponding to the minimum increases with demethylation.

(2) Interactions with the Proton Donor Compounds.

Figs. VII and VIII are representative for the types of curves that were obtained for the interaction of agonists with 4-methyl imidazole and its conjugate acid. In all cases a sharp minimum resulted mainly because of the superposition of a rapidly decreasing polarization contribution upon an even more rapidly increasing overlap component. The electrostatic energy was

generally found to be relatively slowly varying, and the dispersion energy always constituted the smallest contribution to the total energy.

An examination of Fig. IX indicates that the total energy for the interaction of the quaternary methylammonium ion with 4-methyl imidazole is slowly varying. This suggests that the observed sharp minima for the interactions of agonists with the same proton donor compound are primarily due to the interactions with portions of the agonist molecule other than the cationic function, i. e., the "ether" oxygen, and its neighboring groups.

The order of the absolute value of the binding energies for the interaction of the compounds listed in Table II with 4-methyl imidazole (orientation (1)) is

muscarine > methyldilvasene > ACh > methylfurtrethonium >
ethyl choline ether

In each case, with the exception of ACh, the electrostatic energy constitutes the largest contribution to the total interaction energy at the minimum. For all the agents cited, the component energy corresponding to the polarization of 4-methyl imidazole by the agonist amounts to over 80% of the total polarization energy. There is only a small variation in the values for the energy of polarization of the agonist molecules by the proton donor. None of the trends in the component energies for all five compounds appear to correspond entirely to the trend in the binding energies.

The order of the separations of the interacting molecules at the minimum is

ethyl choline ether > methylfurtrethonium > methyldilvasene ~ muscarine > ACh.

The data given in Table III indicates that the order of the absolute value of the total interaction energies at the minimum, for the interaction of the same compounds with 4-methyl imidazole (orientation (2)), is

muscarine > ACh ~ methyldilvasene > methylfurtrethonium > ethyl choline ether.

The fact that the binding energies are larger than those for the previous orientation, shows that a more optimal binding configuration is involved.

For all the agents except ethyl choline ether the polarization contribution to the binding energy exceeds the electrostatic contribution. Both component energies corresponding to the polarization of one molecule by the other are in each case larger than for orientation (1).

The order of the separations at the minimum is

ethyl choline ether > methylfurtrethonium > methyldilvasene > muscarine = ACh

The order of the absolute value of the interaction energies

at a separation of 3 Å, for the interaction of the five agents specified above with the conjugate acid of 4-methyl imidazole (orientation (1)) is (Table IV)

muscarine > methyldilvasene > ACh > methylfurtrethonium > ethyl choline ether

In each case virtually the entire binding contribution is due to the polarization component. Unlike for the interactions with the weak proton donor, the interaction energy resulting from a polarization of the agonists is larger than that due to the polarization of the proton donor compound.

A number of general statements can be made concerning the hydrogen bonding interactions with the agonist molecules.

Despite the differences in the contribution of the various component energies, the order of the absolute value of the binding energies for the interactions with the strong and the weak proton donor compounds (with the latter at two orientations) was found to be

muscarine > methyldilvasene $\gtrsim$ ACh > methylfurtrethonium > ethyl choline ether.

There apparently exists no consistent trend in the component energies at the minimum which holds for all five of the agents investigated. However the binding contributions due to the electrostatic interaction for the first four compounds are always

in the order

muscarine ~ methyldilvasene > ACh > methylfurtrethonium

and the order of the total polarization contribution for the first three compounds is in each case

muscarine ~ ACh > methyldilvasene.

The contribution of the electrostatic component energy relative to the polarization component energy always appears to be largest for the ethyl choline ether.

The separations at the minimum seem to strongly depend on the type of proton donor involved as well as on its orientation. Although it is difficult to generalize, the order of this quantity for the various agents is probably

ethyl choline ether > methylfurtrethonium > methyldilvasene ≥ muscarine ≥ ACh.

(3) Interactions with the Methylammonium Ion.

In their general features the curves for the component energies for the interaction of ACh, muscarine, and methyldilvasene with the methylammonium ion differ from those for the preceding interactions. The curve for the total energy of interaction does not display a minimum, but instead becomes progressively more repulsive with a decrease in the intermolecular separation. This overall repulsion is no doubt due to the electrostatic

interaction between the quaternary ammonium function of the agonists with the methylammonium ion. If one subtracts the energy for the interaction of the latter ion with the quaternary methylammonium ion from the total energy at each point of the interaction energy diagram, a net attractive interaction results. The minima in these curves presumably correspond to the optimum interaction of the agonist portions, other than the quaternary ammonium function, with the methylammonium ion.

The order of the total interaction energies listed in Table V, for the interaction of the three compounds cited above with the methylammonium ion (orientation (1)) is

methyldilvasene ~ ACh > muscarine.

The interaction with muscarine is seen to be least repulsive. However, the order of the absolute value of the same quantities, corrected for the quaternary ammonium function - methylammonium ion repulsion,

ACh ~ methyldilvasene > muscarine.

reveals that the interaction with the muscarine portion other than the cationic group is least attractive.

The trend in the electrostatic component energies parallels that for the total energies. The main contribution to the total polarization energy is due to the polarization of the agonist molecule by the cation. The optimum separation for the interaction with muscarine is approximately 2 Å larger than that for the other

two agents.

The order of the total interaction energies for the corresponding interactions (Table VI) with the methylammonium ion in orientation (2) is

muscarine > methyldilvasene > ACh.

Unlike for the other cases, the interaction energy curve for the interaction with ACh shows that there is a decrease in repulsion with decreasing intermolecular separation. At a distance of 3 Å the net interaction actually becomes attractive.

The trend for the absolute value of the corrected total energies

ACh > methyldilvasene > muscarine

indicates that the attraction of the cation for the agonist portions other than the quaternary ammonium group is greatest in the case of ACh.

Apparently the stronger binding of ACh is due to the fact that for this agent in the series, the electrostatic and overlap contributions are least repulsive while the polarization component is most attractive.

For the interactions with muscarine and ACh, cation orientation (2) leads to a more optimal binding than orientation (1).

(4) Change in Interaction Energy with Rotation.

In the preceding chapter a model for the nicotinic binding compartment was presented. It was suggested that, for an agonist

attached at the anionic anchoring site, a rotation about the quaternary nitrogen might lead to either an optimal binding at the proton donor site (compounds of the first class), or an optimal binding at the cationic site (compounds of the second class). For agents able to interact with both sites, the relative forces of attraction to the receptor binding groups would thus determine the nicotinic activity.

The quantity of interest for an investigation of the change in the binding properties with a rotation away from a given binding group, is the slope of the total interaction energy curve, which is proportional to the torque resulting from the force of attraction that acts on the agonist molecule.

The curves for the change in the interaction energy with a rotation about the quaternary nitrogen center (Figs. XI - XIII) show that, with an increase in the angle of rotation, the electrostatic component becomes more repulsive or less attractive, the overlap component becomes less repulsive, and the polarization component becomes less attractive. In each case the polarization energy constitutes the most rapidly varying contribution. The maximim value of the slope of each total energy curve is given in Table VII.

The order of the slopes for the interactions with ACh is

strong proton donor > cation > weak proton donor.

As was stated above, the same order should also hold for the

relative ability of the respective binding groups to attract the ACh molecule by means of a rotation about the quaternary nitrogen center. The results indicate that for a sufficiently weak proton donor the force of attraction to the cation may be stronger than the force due to the hydrogen bonding interaction.

The order of the slopes for the interactions with methyldilvasene is

strong proton donor > cation ~ weak proton donor.

For this agent the force leading to a binding with the cation is not as great as in the case of ACh. Only for a weak proton donor would the corresponding interaction be able to balance the hydrogen bonding interaction.

The order of the slopes for the interactions with muscarine is

strong proton donor > weak proton donor > cation.

In this case the slope corresponding to the total energy curve for the binding with the cation is actually negative. It appears that a rotation away from the binding group would lead to a slight decrease in repulsion. Consequently the force of attraction to the proton donor would be supplemented by the force due to the cation-agonist interaction.

In summary it may be stated that the order of the three agents investigated, according to the degree to which an interaction with the methylammonium ion is able to outweigh the force of attraction due to the hydrogen bonding interaction, and thus

would lead to a rotation towards the cation, is

ACh > methyldilvasene > muscarine.

VI. RELATIONSHIP OF BINDING AND ACTIVITY

Introduction

According to the model for the AChR active site proposed in Chapter IV the difference in the activities of agonists is related to the ability of these compounds to interact with the receptor cationic and proton donor groups. In the actual binding of a molecule at the active site, the interactions with the binding groups take place simultaneously.

The results cited in the preceding Chapter provide information pertaining to the interaction of agonist molecules with the model systems chosen to represent the postulated receptor components. Since the forces involved are not strictly additive it may be questioned whether it is valid to analyze the receptor binding process on the basis of the findings for the individual interactions. In the following investigation of the relationship between the activity and the agonist-receptor group interactions, it will be assumed that the perturbing effect due to the binding at other sites is not sufficient to alter the order of the binding energies reported above.

It has been proposed that the potency of an agent is related to the stability of the agonist-receptor complex in the equilibrium ionic environment. Two main factors were suggested to be involved in determining the stability: the strength of the binding to the cationic and the proton donor groups, and the degree of the

shielding of the cationic function by suitably bound agonist portions. An examination of the relative importance of both factors, using the calculated results, will now be undertaken.

Role of Binding at Muscarinic Sites

The observed order of the muscarinic activities of the compounds investigated in the previous Chapter is

ACh ≈ methyldilvasene ≈ muscarine ≈ methylfurtrethonium >
ethyl choline ether.

Evidently the trend in the potencies of ACh, muscarine and methyldilvasene is not reflected in either the calculated order of the binding energies for the hydrogen bonding interactions

muscarine > methyldilvasene $\geq$ ACh

or for the interactions with the cationic group

ACh $\geq$ methyldilvasene > muscarine.

The fact that the trends for the two types of binding interactions are the reverse of each other suggests, however, that the sum of the respective binding energies may be approximately the same for each agonist. This would be in agreement with the proposal made in Chapter IV that the muscarinic activity depends on the ability to bind at both receptor groups.

The lower potency of the ethyl choline ether compared to that of methylfurtrethonium may be similarly explained. Methylfurtrethonium is more strongly bound at the proton donor site and presumably also at the cationic site.

There is some question whether it is valid to compare the role that binding plays in determining the activity of agents that do not have the same number of proton acceptor centers. The difference in the stability of the respective agonist-receptor complexes might in part be due to a difference in the free energy of solvation of the compounds involved. For example, methylfurtrethonium would be expected to be a poorer leaving group, in an aqueous environment, than ACh, muscarine, and methyldilvasene.

The binding energy for the binding of methylfurtrethonium at the proton donor site is lower than that of the three agents with which it is equipotent. It appears unlikely that the energy of interaction of the furan ring with the cationic binding group would exceed the energy of interaction of the carbonyl group of ACh with the same site. The sum of the binding energies at both receptor groups would therefore be less for methylfurtrethonium than that of ACh, muscarine and methyldilvasene, and the corresponding agonist-receptor complex might be expected to be less stable.

The apparent lack of correlation between the total energy of binding and the activity in the case of methylfurtrethonium

may be due to the ability of the receptor bound furan ring to protect the cationic group. The oxygen structurally corresponding to the ACh carbonyl oxygen in ACh, muscarine and methyldilvasene, and the conjugated π-system of methylfurtrethonium constitute electron dense regions that could shield the formal positive charge of the receptor group. The lack of a similar feature in the ethyl choline ether molecule might in part be responsible for the low activity of that agent.

Role of Binding at Nicotinic Sites

It was proposed that at nicotinic sites the binding of an agonist at the cationic group serves to promote the activity, while the binding at the proton donor group in most cases diminishes the activity. The nicotinic binding compartment was suggested to differ from the muscarinic compartment in that the angle between the two receptor binding groups is larger. A rotation about the quaternary nitrogen would be required to bring the molecule from the optimum position for binding at one group to the optimum binding position at the other group. Agents of the first class would be bound at the proton donor site, while agents of the second class would interact more strongly with the cationic site.

The observed order of the nicotinic activities of the agents for which calculations were performed is

ACh > methyldilvasene ~ ethyl choline ether > muscarine > methylfurtrethonium

(measured on the frog rectus preparation [99, 110])

ACh > methyldilvasene = methylfurtrethonium > muscarine

(measured on the cat blood pressure [99]).

Clearly the two sets of data do not indicate the same relative potencies for muscarine, methyldilvasene, and methylfurtrethonium.

It is noteworthy that in both cases the order

ACh > methyldilvasene > muscarine

was reported. The same trend for these three agents is also reflected in the calculated energies of binding at the cationic receptor group. The reverse trend is found for the energies of binding at the proton donor group. This indicates that the calculated results are in agreement with the proposal that binding at the cationic site promotes nicotinic activity, while binding at the proton donor site diminishes the activity.

The high activity of agents that preferentially bind at the cationic site may again be attributed to their suggested role in protecting that receptor group from the ionic environment. It is significant that the trend in the nicotinic potencies of ACh, muscarine and methyldilvasene also parallels the trend in the degree to which the force of attraction of each compound to the cationic group is likely to outweigh its attraction to the proton donor group.

It was found that for the two agents, ACh and methyldilvasene, there may be a net force of attraction to the cationic site. The

difference of the respective activities might therefore primarily be due to the difference in the binding energies at that receptor group.

The activity of the agents that are expected to be more optimally bound at the proton donor site, i. e. muscarine, methylfurtrethonium, and the ethyl choline ether, is presumably due to their ability to protect the cationic site, in spite of their less favourable orientation. The factors that would determine the potency of these compounds are: the strength of the binding, the electron density of the portion of each molecule facing the cationic receptor group, and the distance of these portions to the receptor group.

On the basis of the first two factors muscarine would be expected to be more potent than methylfurtrethonium and the ethyl choline ether. However the order of the binding separations for the hydrogen bonding interactions was calculated to be

ethyl choline ether > methylfurtrethonium ≥ muscarine.

Perhaps the relatively large activity of the ether, measured on the frog rectus preparation, is due to its more favourable orientation to the cationic group, resulting from a greater distance to the proton donor group.

The discrepancy in the relative magnitudes of the nicotinic potencies of muscarine and methylfurethonium will be discussed further below.

Mechanism of Action

The discussion in the preceding sections has shown that the calculated results lead to predictions concerning the mode of action of agonists that are in agreement with the mechanism postulated in Chapter IV. It was proposed that the characteristics of the agonist-receptor group interactions determine both the thermodynamic as well as the kinetic stability of the agonist-receptor complex.

In this section the details of the mechanism that was suggested to be involved in the production of a pharmacological response will be elaborated.

(1) Steps in the Mechanism

It is possible to give the following schematic description of the specific molecular events that lead to the agonist-induced opening and the closing of ionic channels in the membrane

$$\begin{tikzcd}
AB + E_{+} \arrow[r] & B + AE_{+} + R \arrow[rr, bend left, "\text{pore opening}"] & & ARE_{+} \arrow[d] \\
AB + E_{\pm} & B + AE_{\pm} + R \arrow[l] \arrow[u] & & ARE_{\pm} \arrow[ll, bend left, "\text{pore closing}"]
\end{tikzcd}$$

where R represents the receptor, A denotes an agonist molecule, E_{+} and $E_{\pm}$ represent respectively the immediate receptor environment before and after the ion exchange process through the pore have taken place, and B stands for the bulk solution in the synaptic cleft.

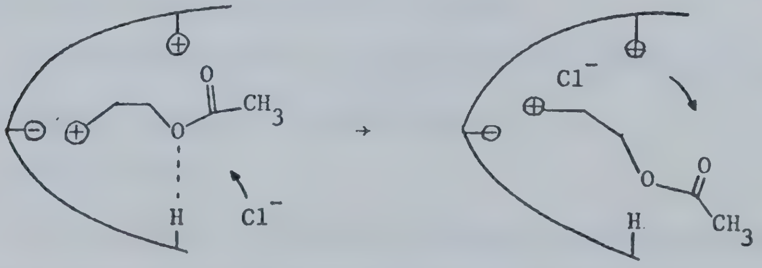
The presence of the agonist in the bulk solution (AB) leads to the diffusion of some agonist molecules into the portion of the solution in the neighborhood of the receptors (AE_+), which contains certain ions at non-equilibrium concentrations. The interaction of the agonist with a receptor and/or its immediate environment (Watkins' mechanism) causes the opening of a pore and the initiation of the irreversible ion exchange processes across the membrane. As the ionic environment changes the agonist-receptor complex (initially (ARE_+)) becomes progressively less stable ($ARE_\pm$). Under the perturbing influence of the surroundings the complex eventually dissociates, which leads to the closing of the pore. The irreversible action of the sodium pump serves to reestablish the initial concentration gradient across the membrane. Diffusion into the bulk solution, and in the case of ACh inactivation by AChE, are responsible for the subsequent removal of agonist molecules from the receptor surroundings.

(2) The Life Time of a Pore

It was proposed that the strength of the response to a given agent is related to both the number and the average life time of the pores that are opened. Since even the small alkyl-trimethylammonium ions are agonists, it seems that the portion of the agonist molecules involved in the opening of a pore is the cationic group. In the case of most potent agents this will either be a quaternary or a tertiary ammonium group. It appears

therefore that the observed difference in the activities of agonists is not attributable to a difference in the ability to open a pore. The average number of pores that are opened at a given concentration will be approximately the same for all agents containing a suitable cationic function. The magnitude of the response is thus presumably proportional to the average life time of a pore, which depends upon the stability of the agonist-receptor complex.

It may be concluded that the open pore configuration will be maintained as long as the agonist-receptor complex is intact, and that the rate of the closing of a pore is consequently equal to the rate of the disruption of the complex. Since the latter process involves the departure of a positive ion, the presence of an anionic species could constitute a stabilizing influence. The attraction of a chloride ion to the surplus positive charge of the receptor cationic group could serve to initiate the disruption of the complex and the anion might then facilitate the departure of the agonist molecule from the active site, e. g., in the case of ACh



One may propose that two factors are of importance in determining the kinetic stability of the agonist-receptor complex: (a) The degree to which the receptor environment is polarized by the effective charge of the receptor cationic group - which determines the probability that an anion will approach the complex. (b) The magnitude of the force that constrains the agonist molecule at the receptor group - which determines the kinetic energy that an anion must possess to sufficiently perturb the complex, and thus to initiate its disruption.

Only the former factor would be affected by the ion exchange processes that take place through the pores. A change in the ionic concentrations could alter both the probability of finding an anion in the vicinity of the receptor, as well as the energy required to remove an anion from its ionic environment in the solution.

Mechanism for Synaptic Transmission

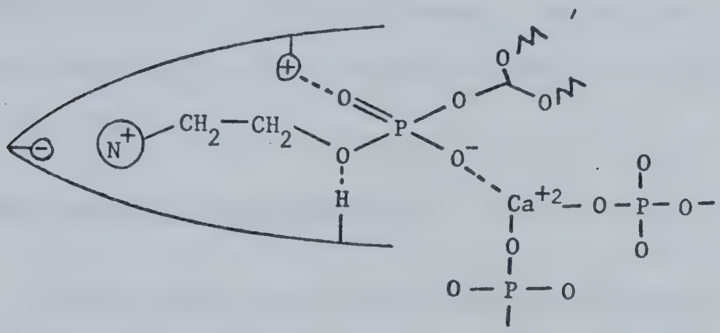
On the basis of the preceding discussion it is possible to offer an extension of Watkins' mechanism, that is particularly suited for explaining the experimental findings pertaining to the excitation of natural membranes by ACh.

The following steps are suggested to be of importance in the process of synaptic transmission:

1) Resting State.

As in the case of the pore forming proteins, the presence of the AChR protein in the membrane may lead to the formation of

a permanent pore. In the resting state an extended calcium ion - phospholipid complex might span the surface of the membrane, blocking the access of monovalent ionic species to the pore. In particular, the binding of the polar portion of phosphatidylcholine at the active site of the AChR might provide an anchoring site for Ca^{+2}, and thus promote the formation of the complex in the vicinity of the receptor



2) Pore Opening

The role of ACh could be to compete with Ca^{+2} for binding at the negatively charged phosphate group. This would also entail the "dissolving" of the complex and thus the opening of the pore to monovalent ions.

3) Binding of ACh

It may be postulated that the ability to participate in the complex serves to stabilize the receptor-lipid association. The approach of an ACh molecule would lead to the displacement of the lipid polar portion from the receptor binding compartment, and to the binding of the transmitter compound.

4) Displacement of ACh

As the ionic processes taking place through the pore reach completion the ionic environment of the receptor changes. The exchange of sodium ions leads to a reduction in the concentration of NaCl in the vicinity of the receptor. Since monovalent cations are believed to destabilize a Ca^{+2}-phospholipid association, the formation of a complex would be favoured upon the completion of the exchange processes. The approach of anions could serve to displace the agonist, and thus initiate the closing of the pore.

Construction of the Active Site

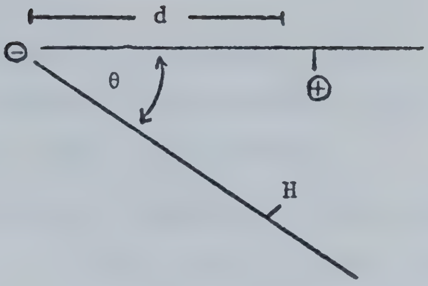
It was already noted that aside from the distinction between muscarinic and nicotinic sites, cholinergic receptors in diverse tissue preparations also differ in the relative amounts of a given agent that are required to produce a standard response. Thus the structure activity relationship for muscarinic receptors appears to be very strict at receptors in the frog heart, and less strict at those in the guinea-pig ileum. Similarly the requirements for activity are more stringent at the nicotinic receptors in the frog rectus preparation than at those involved in producing the cat blood pressure response.

These observed differences in potency are presumably not attributable to factors such as the non-specific absorption of the agents at neighboring sites, but are directly related to

a difference in the construction of the respective receptor sites. In this context it may be worth noting that, by virtue of its pore-forming function, the AChR is presumably closely associated with the non-polar portions of the membrane lipid. Since the physical properties of the membranes in various tissues are known to vary with the amount of cholesterol and proteins they contain, it may be surmised that the environment of the receptor in the membrane could affect the tertiary structure of the receptor protein to some extent. A slight change in the relative position of the binding groups at the active site could lead to a change in the binding properties of the receptor.

The results of the quantum mechanical calculations reported in the last section show that the energy of binding of ACh at the cationic receptor group is particularly dependant upon the position of the binding group. Since potencies are generally expressed relative to the standard response to ACh it may be proposed that the position of the cationic group relative to the other receptor groups is of great importance in determining the structure activity relationship at a given receptor.

The observed differences in activity, for a receptor of a given type in various tissues, could be due to variations in both the angle θ as well as the distance d


d
θ
H

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